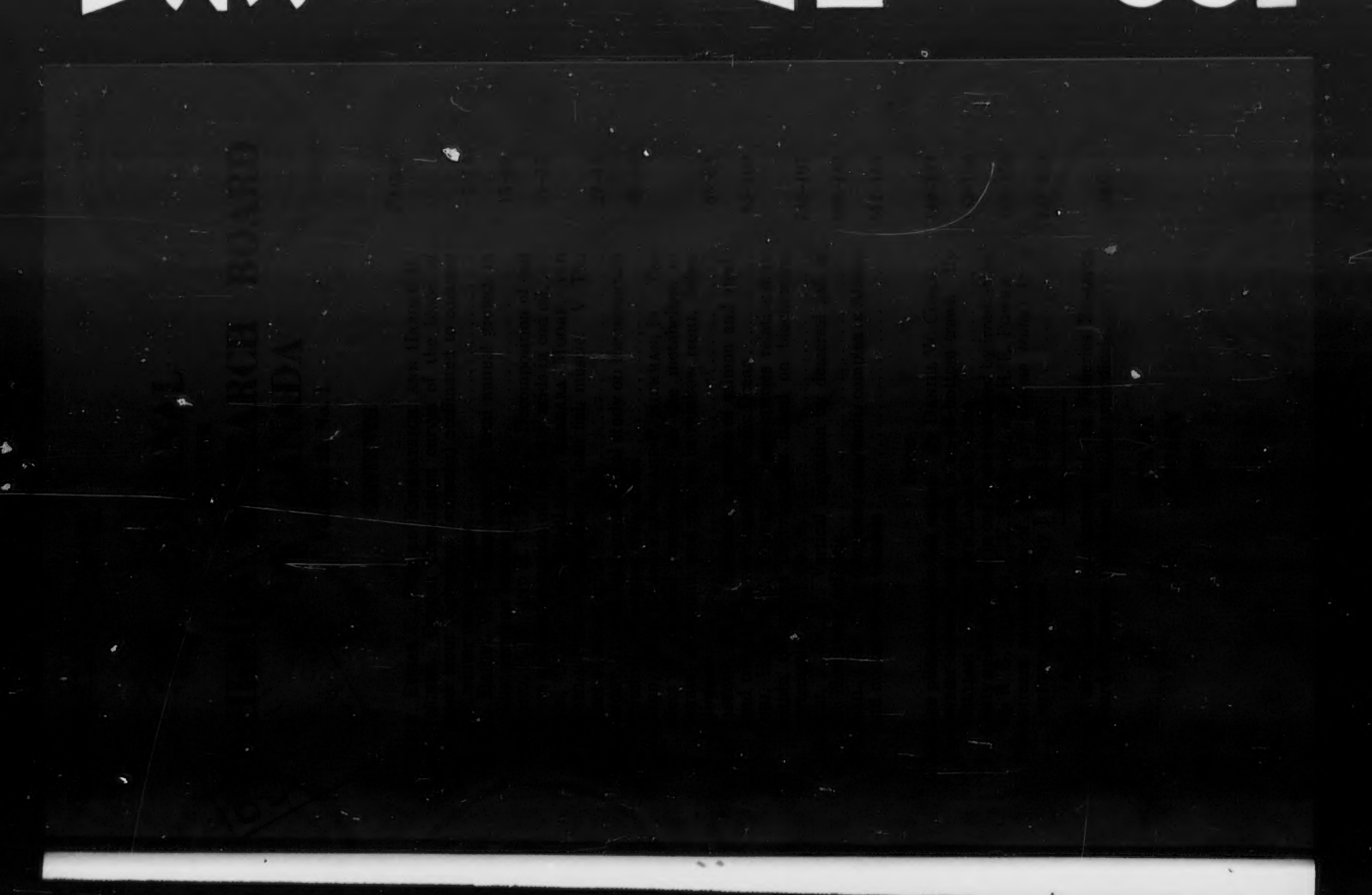

Oxygen Dissociation Curves of the Blood of Brook Trout (*Salvelinus fontinalis*) Acclimated to Summer and Winter Temperatures[1]

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ABSTRACT

The respiratory function of the blood of brook trout, acclimated to summer and winter conditions, was investigated at various temperatures and at various tensions of carbon dioxide. The marked sensitivity of hemoglobin to carbon dioxide served to offset the influence of lower temperature upon the equilibrium of oxygen and hemoglobin. There did not appear to be any adaptation of the Bohr effect to differences in acclimation temperatures.

INTRODUCTION

Bohr et al. (1904) demonstrated that carbon dioxide reduces the affinity of mammalian hemoglobin for oxygen. This influence is today known as the Bohr effect. Barcroft and Hill (1910) showed that an increase in temperature reduced the affinity of hemoglobin for oxygen. Krogh and Leitch (1919) investigated the respiratory function of blood in fishes. They discovered that the disadvantage conferred by lower temperatures to favourable unloading tensions of oxygen from hemoglobin in the tissues was, in part, compensated by a marked increase in the sensitivity of hemoglobin to an increased tension in carbon dioxide. Root (1931) studied marine fish blood in greater detail and found that certain fishes exhibited a very marked Bohr effect. Black (1940) reported a gradation in the Bohr effect in four species of freshwater fish of the temperate zone, and that this gradation appeared to be related to differences in the environmental niche held by these species in the summer. Fry et al. (1947) studied the influence of temperature on the asphyxiation of young goldfish (*Carassius auratus* L.) under various tensions of oxygen and carbon dioxide. They found there was a marked increase in the sensitivity of the goldfish to carbon dioxide asphyxiation as the temperature of acclimation was decreased. They attributed this increase in sensitivity to the effect of temperature on both the mechanical transport of blood and the chemical transport of oxygen by the blood.

The present investigation was undertaken to determine whether temperature acclimation exerted an effect on the equilibrium between oxygen

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and hemoglobin in the presence of carbon dioxide and also to describe in greater detail the function of blood in brook trout (*Salvelinus fontinalis*).

METHODS

The brook trout (*Salvelinus fontinalis*) were raised at the principal trout raising station in inland Nova Scotia, operated by the Fish Culture Branch of the Department of Fisheries, Ottawa, Canada. The fish were transferred to Bedford hatchery at Bedford, Nova Scotia, for holding and for taking blood samples. Most of the fish were 2 years old, but, in certain cases 3-year-old trout were also used.

The environmental water temperature at the Bedford fish hatchery, which determined the history of acclimation, ranged from 0 to 7 C in winter and from 7–21 C in summer.

The methods used have been described by Black (1940) and by Ferguson and Black (1941). The analytical procedures were those described by Peters and Van Slyke (1932).

The samples were transported on ice to the Department of Physiology at Dalhousie University, Halifax, for analysis. The journey occupied 1 hr. It is unlikely that the time taken for sampling and transport caused any great change in metabolism in the collected blood samples. Ferguson and Black (1941) found that the highest metabolic rate for rainbow trout (*Salmo gairdneri*) kept on ice, was 0.5 ml of oxygen per 100 ml of blood per hour.

In several cases it was necessary to pool samples of blood from two or more fish for construction of the oxygen dissociation curves. Samples from single fish were used for the construction of carbon dioxide dissociation curves incidental to estimating the various gas tensions. Due to the difficulty in obtaining blood, it was necessary to use an excess of heparin solution to arrest clotting.

RESULTS

Table I shows oxygen capacities and hematocrits as taken, and oxygen capacities and hematocrits corrected for quantity of heparin used per pooled sample of blood, obtained from trout acclimated to summer and winter conditions. As noted in the Table, these samples were used for the construction of the oxygen dissociation curves at 0 C. Only in one case, namely the winter at a temperature of 0 C were the oxygen capacity and hematocrit values equal to those reported by Irving et al. (1941) and Field et al. (1943) for the same species. While some of the fish used in this present study were parasitised by gill copepods, only one such fish was actually included in the values for the winter at 0 C (1946). No explanation is offered for the spread of lower values found for most of the fish in this study.

In Table II, the average oxygen capacity, the average red cell volume, and the average oxygen capacity of 100 ml of cells, for observations made during summer and winter conditions are presented. Inasmuch as the average

TABLE I. Number of fish used per pooled sample of blood, quantity of blood, oxygen capacity, red cell volume, quantity of heparin, and oxygen capacity and red cell volume corrected for heparin dilution in trout acclimated to summer conditions and winter conditions.

Season	Temperature	Number of fish per pooled sample	Quantity of sample	Oxygen capacity	Red cell volume	Heparin	Corrected oxygen capacity	Corrected red cell volume
			ml	%	%	*ml*	%	%
		2	12	6.76	17.1	1.0	7.37	18.7
Summer	0 C	1	5.5	7.32	23.5	0.5	8.05	25.9
		3	14.0	6.29	17.9	1.0	6.77	19.3
		3	10.0	7.07	21.8	1.25	8.08	24.9
	Average:	2.25	10.38	6.86	20.08	0.94	7.58	22.2
	Range:		5.5–14.0	6.29–7.32	17.1–23.5	0.5–1.25	6.77–8.08	18.7–25.9
	Standard error:			0.222	1.535		0.312	1.863
		1	7.0	9.36	28.2	0.5	10.08	30.4
Winter	0 C	1	8.0	10.84	30.5	0.75	11.96	33.7
		1 (Parasitised)	7.0	8.18	25.4	0.75	9.16	28.5
	Average:	1	7.33	9.46	28.03	0.67	10.4	30.9
	Range:		7.0–8.0	8.18–10.84	25.4–30.5	0.5–0.75	9.16–11.96	28.5–33.7
	Standard error:			0.770	1.491		0.824	1.519

TABLE II. The combined summary of oxygen capacity and red cell volume for brook trout blood, corrected for heparin dilution, and the oxygen capacity of 100 ml red blood cells.

Season		Number of determinations	Oxygen capacity	Red cell volume	Oxygen capacity 100 ml cells vol % HbO_2
Summer	Average ± S.E.:	14	7.470±0.424	22.214±1.278	33.79±0.974
	Range:		5.10–10.65	14.6–31.2	28.72–42.86
Winter	Average ± S.E.:	18	8.116±0.373	22.994±1.123	33.45±0.617
	Range:		5.82–11.96	17.2–33.7	31.81–42.01
Combined seasons	Average ± S.E.:	32	7.831±0.281	22.653±0.833	34.73±0.559
	Range:		5.10–11.96	14.6–33.7	28.72–42.86

oxygen capacities for summer and winter do not differ significantly ($0.221 < t_{.05}$ df 30), the combined averages are also presented.

OXYGEN DISSOCIATION CURVES

0 C

Oxygen dissociation curves were constructed for blood drawn from fish equilibrated for summer and winter conditions. Oxygen dissociation curves were constructed for three tensions of carbon dioxide, namely, 0–1 mm Hg, 10 mm Hg, and 40 mm Hg. These results are illustrated in Fig. 1. No distinction

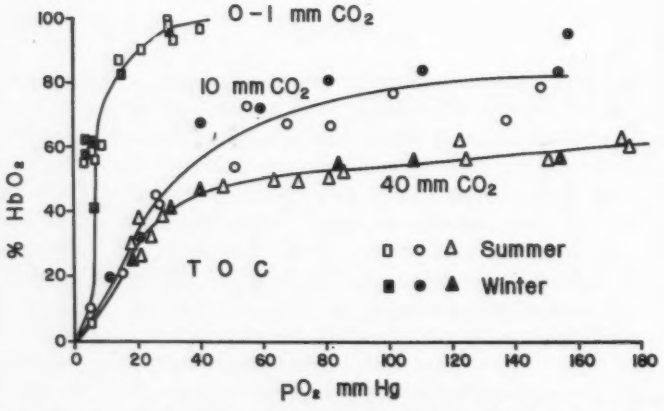

FIG. 1. Oxygen dissociation curves for brook trout blood acclimated to summer and winter conditions at various tensions of carbon dioxide, equilibrated at 0 C.

could be made between the data for summer and winter conditions, consequently a single S-shaped curve was drawn through the results for pCO_2, 0–1 mm Hg.

To facilitate later discussion the tension of unloading (pO_2 for 50% HbO_2) is presented in Table III. As will be noted shortly the tension of loading denoted by Krogh and Leitch (1919) as pO_2 for 95% HbO_2, cannot be given because of the sensitivity of the hemoglobin to carbon dioxide. Data for pCO_2 10 mm Hg and 40 mm Hg, are also illustrated in Fig. 1 and summarised in Table III.

TABLE III. Tensions of unloading ($t\mu = pO_2$ for HbO_2 of 50%) for oxygen, in brook trout (*Salvelinus fontinalis*) blood equilibrated at five different temperatures and three different tensions of carbon dioxide.

Temperature (C)	0–1 mm Hg pCO_2	10 mm Hg pCO_2	40 mm Hg pCO_2
0	7	33	58
5	5	26	59
15	12	43	142
20	17	40	84
25	21	54	180

5 C

Oxygen dissociation curves for fish acclimated to winter conditions only were constructed (Fig. 2, Table III). The study was interrupted before data could be obtained for fish acclimated to 5 C with summer conditions prevailing.

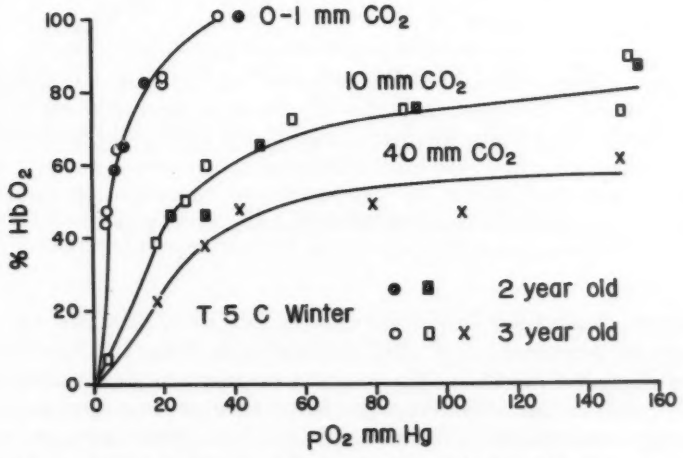

FIG. 2. Oxygen dissociation curves for brook trout blood acclimated to winter conditions at various tensions of carbon dioxide, equilibrated at 5 C.

15 C

Oxygen dissociation curves for trout acclimated to summer and winter conditions are shown in Fig. 3 and Table III. There did not appear to be any significant difference between summer and winter data for low carbon dioxide tension and high carbon dioxide tension. Above pO_2 60 mm Hg, however, blood taken from fish acclimated to summer conditions was consistently less sensitive to carbon dioxide at the 10 mm Hg tension of carbon dioxide. However, since only two data were obtained for winter conditions a single curve has been drawn for all the data.

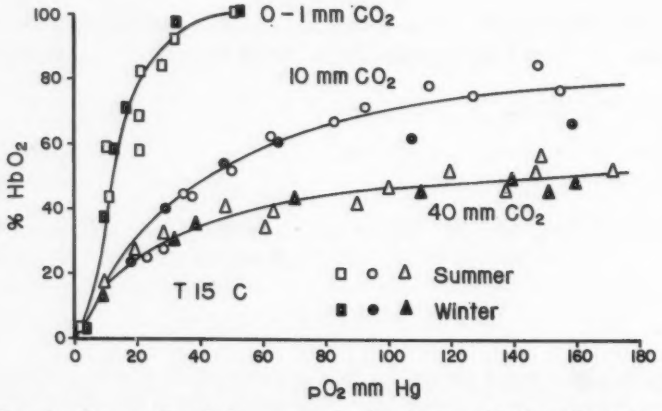

FIG. 3. Oxygen dissociation curves for brook trout blood acclimated to summer and winter conditions at various tensions of carbon dioxide, equilibrated at 15 C.

20 C

Oxygen dissociation curves are presented in Fig. 4 and summarised in Table III for fish acclimated to summer conditions at three different tensions of carbon dioxide. This study was terminated before data could be obtained for fish acclimated to winter conditions. While no data were obtained at tensions of oxygen lower than 30 mm Hg and percentage saturation less than 20, the beginning of each curve is represented as showing an S-shape.

25 C

Oxygen dissociation curves for fish acclimated to summer and winter conditions are presented in Fig. 5 and summarised in Table III. As was the case for data presented for 15 C (Fig. 3), data for low and high carbon dioxide tensions are consistent. However, there is a marked scattering of data for the curve constructed at a pCO_2 10 mm Hg. In Fig. 3, the Bohr effect seemed to be greater for fish acclimated to winter conditions whereas in Fig. 5, the Bohr effect appeared greater for summer conditions for oxygen dissociation curves

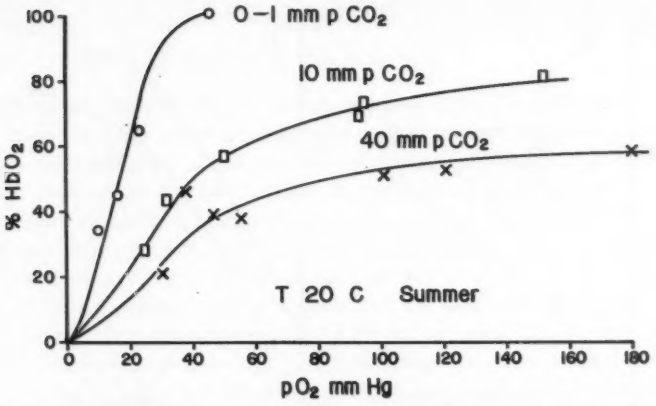

FIG. 4. Oxygen dissociation curves for brook trout blood acclimated to summer conditions at various tensions of carbon dioxide, equilibrated at 20 C.

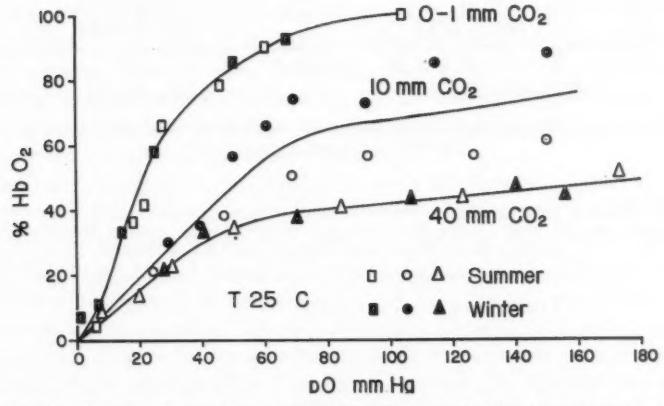

FIG. 5. Oxygen dissociation curves for brook trout blood acclimated to summer and winter conditions at various tensions of carbon dioxide, equilibrated at 25 C.

equilibrated to a pCO_2 10 mm Hg. Nonetheless a single curve has been drawn for blood acclimated to summer and winter conditions for each carbon dioxide tension.

CARBON DIOXIDE DISSOCIATION CURVES AND VENOUS BLOOD GASES

Incidental to determining the tension of venous blood gases, carbon dioxide dissociation curves were prepared at 0 C from fish which had been acclimated to winter conditions (Fig. 6, Table IV). The study was terminated before results could be obtained for trout which had been acclimated to summer conditions.

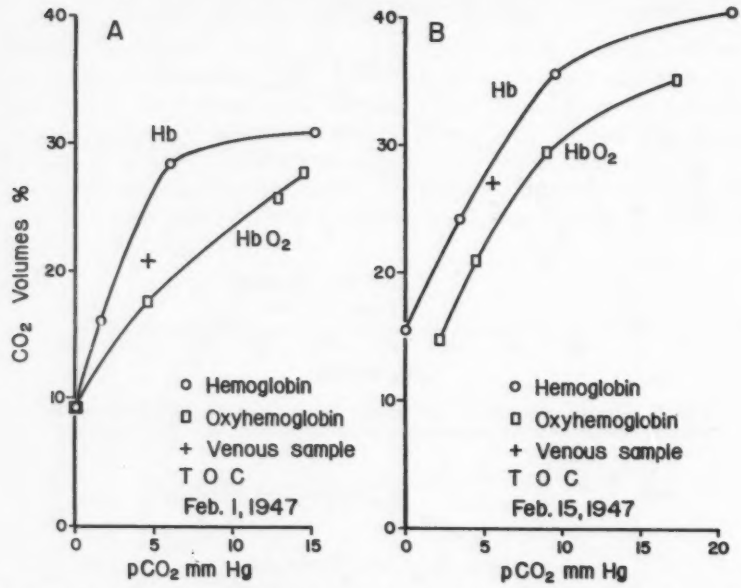

FIG. 6. Carbon dioxide dissociation curves for brook trout blood acclimated to winter conditions, and equilibrated at 0 C.

TABLE IV. Venous blood gases for rainbow trout (*Salmo gairdneri*) acclimated to summer conditions and brook trout (*Salvelinus fontinalis*) acclimated to winter conditions. Data for rainbow trout taken from Ferguson and Black (1941).

Species	Temperature	CO_2	HbO_2	pCO_2
Rainbow trout	*C*	*vol %*	*%*	*mm Hg*
B	22 summer	21.0	0	9.0
C	22 summer	24.8	3	10.0
D	15 summer	19.4	0	8.0
E	15 summer	22.8	0	10.0
Brook trout				
A	(0–1) winter	21.6	33	6.5
B	(0–1) winter	27.0	33	5.6

While the data obtained on February 15, 1947 (Fig. 6b) are more consistent than those of February 1, 1947 (Fig. 6a), both sets of curves are presented. In both instances the venous oxygen saturation was one-third (Table IV) and carbon dioxide tension averages 6 mm Hg for both observations.

DISCUSSION

Oxygen Capacity and Erythrocyte Volume

Influence of the pooling of blood samples and the addition of heparin solution upon the hematocrit readings and oxygen capacities is shown in Table I. In Table I all details are given for four separate samples of blood. During the summer in most cases it was necessary to pool blood from two or more fish in order to obtain enough blood to construct the oxygen dissociation curves, while in the winter only one fish was required in each of the three experiments. No explanation is offered for the differences observed.

The average oxygen capacity corrected for heparin dilution is 7.47 vol per cent for summer conditions and 8.116 vol per cent for winter conditions (Table II). Since there was no significant difference between these two averages ($0.221 < t_{.05}$ for df 30) an average for all the oxygen capacities for both single and pooled fish is 7.83 vol per cent. This is less than the average of 11.7 vol per cent reported by Irving et al. (1941) and 11.56 vol per cent derived from the data of Field et al. (1943). Only in one season, namely winter 1946, was the oxygen capacity close to the averages reported in the other papers (10.84, Table I, winter 0 C). Parasitism by gill copepods cannot be given as a reason for lower oxygen capacity since following 1946 mainly 2-year-old trout were used, an age group not yet impaired by gill parasitism. In the single instance where a parasitised fish was used, the oxygen capacity was higher than the average for winter acclimated trout (Table II).

Hematocrit readings corrected for heparin dilution are shown in Tables I and II. The average for all values was 22.65% from the data of Irving et al. (1941) and the average of 27.2% given by Field et al. (1943). The oxygen capacity of 100 ml of red blood cells was found to be 34.73% (Table II), an average which is lower than that of 40% given by Irving et al. (1941).

Hematocrit values were higher in summer. These higher values may have been due to an increase in the number of circulating erythrocytes by production or by release from storage sites. Plasma water may also have been transferred osmotically to muscle associated with the known increased activity of trout in summer (Swift, 1962).

OXYGEN DISSOCIATION CURVES

The data for various oxygen dissociation curves at different temperatures are summarised in Table III and illustrated in Fig. 1–5.

pCO_2, 0–1 mm Hg

At a pCO_2, 0–1 mm Hg, the tension of unloading of oxygen increases from 7 to 21 mm Hg at 25 C. These findings show the same rate of change at a slightly lower affinity than the results obtained by Irving et al. (1941, p. 8).

Data for blood taken from fish acclimated to summer and winter conditions are shown in Fig. 1, 3, and 5. Examination of this information reveals no consis-

tent difference between summer and winter conditions. Accordingly one curve has been drawn for all the data at a pCO_2 0–1 mm Hg for temperatures 0–1, 15, and 25 C.

pCO_2, 10 mm Hg

Curves are presented in Fig. 1–5, and results summarised in Table III. In general the effect of carbon dioxide is of the same order as that reported by Irving et al. (1941), namely, there is a very pronounced Bohr effect as first noted by Krogh and Leitch (1919).

The combined effect of temperature and carbon dioxide, although variable, when compared with the data reported by Irving et al. (1941 p.8) reveals the same order of change.

Part of the variability in the results can be correlated with summer and winter equilibration. The curves constructed for 0 C and 25 C (Fig. 1 and 5) appear to reveal that summer acclimated blood is more sensitive than winter. For blood equilibrated at 15 C the reverse effect was shown, namely, blood acclimated to winter conditions was more sensitive to carbon dioxide. Admitting that the results are limited in number (for two data only), the conclusion was reached that there was no consistent effect of acclimation upon the Bohr effect.

Powers et al. (1932) encountered variability in the Bohr effect in fish blood, which variability they attributed to equilibration at different sides of the iso-electric point of hemoglobin. Riggs (1960) found that the Bohr effect is partly dependent on the number of sulfhydryl groups present in the hemoglobin molecule. Judging preliminary observations by von Buddenbrock (1938) and by Auvergnat and Secondat (1942), it is possible that different levels of lactate may also have influenced the sensitivity of the hemoglobin to carbon dioxide, although Riggs (1960) shows that the oxygen affinity was depressed in the presence of carbon dioxide even when measured at a constant pH. If a difference in the Bohr effect is to be attributed to a difference in acclimation, the factors enumerated by Powers et al. (1932) and Riggs (1960) would have to be investigated.

In taking blood samples more heparin was used for fish acclimated to summer conditions, and pooling of samples was usually resorted to during this season. While it is unlikely that these two factors affected variability, it is also true that their possible importance was not measured.

pCO_2, 40 mm Hg

In Fig. 1–5, the Bohr effect at a pCO_2 of 40 mm Hg is illustrated. The tension of unloading for various temperatures is given in Table III. At a pCO_2 of 40 mm Hg the Bohr effect is so great that even at an equilibration temperature of 0 C the hemoglobin is only 60% saturated at a pO_2 180 mm Hg. This order of sensitivity was first noted in marine fish blood by Root (1931).

The variability of the Bohr effect is not very great for any one curve, yet Fig. 3 and 4 and Table III reveal that the Bohr effect at an equilibration tempera-

ture of 20 C is less than that at 15 C. It is possible that since the curves were constructed at any one equilibration temperature mainly on a single sample of blood, the Bohr effect at 20 C may have been depressed by the presence of endogenous lactate produced during possible struggling while obtaining the sample. Von Buddenbrock (1938) believed from his experiments that the endogenous production of lactate through the struggling of fish in captivity caused the deoxygenation of blood observed in the gills. However, no measurements of blood lactates were made in this present study.

Examination of the oxygen dissociation curves constructed at various carbon dioxide tensions and equilibrated at 0–1, 15, and 25 C for blood taken from brook trout (*Salvelinus fontinalis*) acclimated to summer and winter temperatures reveals no consistent correlation of the Bohr effect with the temperature of acclimation. The suggestion was advanced by Fry et al. (1947) that changes in the sensitivity to carbon dioxide in acclimated fish lies possibly in the effects of temperature on both mechanical and chemical transport of oxygen by the blood. The chemical explanation is not supported by the data presented in this paper.

Hoar (1955) found that the seasonal variations in resistance of goldfish to temperature were related more to total periods of exposure to light than to acclimation to summer or winter temperatures. In the present experiment low temperatures were associated with the shorter light period of winter and the periods of warm temperature were associated with the longer light periods of summer.

CARBON DIOXIDE DISSOCIATION CURVES

Incidental to the determination of physiological gas tensions, carbon dioxide dissociation curves were carried out on two trout during the winter of 1947 (Fig. 6). Of the two curves, the data for the second trout investigated upon February 15, 1947, are more in keeping with the curves obtained by Ferguson and Black (1941) for rainbow trout acclimated to summer conditions. However, the carbonate level for a given tension of carbon dioxide and the degree of oxygenation is twice as great in brook trout at 0 C than in rainbow trout studied at 15 C.

The Haldane effect, i.e. the influence of oxygenation of the hemoglobin, reducing the carriage of carbon dioxide, is considerable for brook trout and much greater than that observed by Ferguson and Black (1941) for rainbow trout acclimated to summer conditions.

PHYSIOLOGICAL GAS TENSIONS

The physiological gas tensions have been derived from the two samples of blood used for the carbon dioxide dissociation curves (Fig. 6). These values have been compared with the data from rainbow trout derived from Ferguson and Black (1941).

As denoted in Table IV, both samples of venous blood taken from brook trout, revealed a degree of oxygenation of 33%. The carbon dioxide content of

the venous blood was 21.6 vol per cent for the first sample and 27.0 vol per cent for the second. Data on respective graphs reveal that the pressure of carbon dioxide for the first sample should be 6.5 mm Hg and the second sample 5.6 mm Hg and an oxygen tension of 15 mm Hg. These actual tissue tensions would undoubtedly show a carbon dioxide value higher than 6 mm Hg and an oxygen tension lower than 15 mm Hg. The approximations are for averages resulting from the mixing of blood distributed via the gills.

The carbon dioxide tension estimated by Ferguson and Black (1941) in Table IV for rainbow trout acclimated to summer conditions averaged 9 mm Hg and the venous samples were practically devoid of oxygen. Admitting that there is the question of the difference of species between the two studies, none the less the data from the two are concordant. Carbon dioxide tension is higher in summer conditions and the oxygen tension is higher during the winter reflecting obvious increased metabolic activity at the higher temperatures.

From Fig. 1, 3, and 5 and from Table IV, it is possible to arrive at an approximation of the physiological oxygen dissociation curves for 0–1, 15, and 25 C. During winter, the loading of oxygen in the gills at 15% saturation would be a pO_2 of 30–40 mm Hg and a pCO_2, 0–1 mm Hg; the tension of unloading or pO_2 at 50% HbO_2 would be 25 mm Hg in the presence of pCO_2 tension of 6 mm Hg. At 15 C, the tension of loading would be a pO_2 of 40 mm Hg, and the tension of unloading at a pCO_2 of 9 mm Hg would likewise be a pO_2 of 40 mm Hg. At 25 C, the tension of loading would be depressed to a pO_2 of 50 mm Hg at a pCO_2 of 0–1 mm Hg; the tension of unloading at a pCO_2 of 9 mm Hg would be a pO_2 of 50 mm Hg. These results bear out the claim of Krogh and Leitch (1919) that the increased Bohr effect in trout blood serves to overcome the adverse effect of lowered environmental temperature at the unloading phase of the respiratory cycle.

SUMMARY

Oxygen dissociation curves were constructed for brook trout blood acclimated to winter conditions at 0, 5, 15, and 25 C and at pCO_2 levels of 0–1 mm Hg, 10 mm Hg, and 40 mm Hg.

Oxygen dissociation curves were constructed for brook trout blood acclimated to summer conditions at 0, 15, 20, and 25 C and at pCO_2 levels of 0–1 mm Hg, 10 mm Hg, and 40 mm Hg.

Carbon dioxide dissociation curves were constructed at 0 C for two samples of brook trout blood acclimated to winter conditions.

Venous gas tension in two samples of blood, acclimated to winter conditions only, was estimated to be a pCO_2 of 6 mm Hg and a pO_2 of 15 mm Hg at 0 C.

Carbon dioxide was shown to have a marked effect in depressing the affinity of hemoglobin for oxygen, i.e. a large Bohr effect.

Oxygenation of the blood reduced the combining power of the blood for carbon dioxide, i.e. the Haldane effect.

Magnitude of the Bohr effect was not altered by acclimation from summer to winter temperature.

ACKNOWLEDGMENTS

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Dependence of Total Annual Growth in Yellow Perch on Temperature[1,2]

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ABSTRACT

Total yearly growth of female yellow perch, *Perca flavescens* (Mitchill), in South Bay, Lake Huron, estimated by the distance along the lateral radius from last annulus to scale edge, showed a marked relationship to mean water temperature at a depth of 20 ft. When published data on growth of perch in Lower Red Lake, Minnesota, were compared with mean air temperatures for the period June through September at Redlake, no such relationship could be demonstrated. The reasons for the different results obtained in these and in other studies could not be determined.

INTRODUCTION

It is probable that growth of fish in the wild is affected by a variety of factors which may interact in numerous ways. Such factors as the presence of pollutants, heavy parasite loads, space limitations and others, may influence growth. On the other hand, factors such as environmental temperature and the quantity and quality of the food supply would always be of importance to growth of fish. This paper reports on a study of the effects of temperature on total annual growth of yellow perch, *Perca flavescens* (Mitchill). There have been a few other studies of growth–temperature relationships with perch, the results of which are discussed in relation to those reported here.

METHODS AND MATERIALS

Growth was studied by means of scale measurements of perch caught in the outer basin of South Bay, a 16-mile long arm of Lake Huron extending into the southeast corner of Manitoulin Island (Fraser, 1955; McCombie, 1959). The analysis was confined to females because of the difficulty in determining ages from scales of male perch. Scales and accompanying pertinent information from perch caught from 1958 to 1962 inclusive, and in 1964 were used. Scales were taken from the side of the fish below the lateral line and above the tip of the pectoral fin. Most of the fish were caught in five pound nets (about 7% were taken by anglers) fished in the outer basin of South Bay. Mesh size (stretched measure) in the pots of two nets was 1 inch, in two others, 1.5 inches, and in the fifth, 3 inches.

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Growth was estimated from scales taken from some 675 fish captured in spring of each year before seasonal growth began. Cellulose acetate slides bearing scale impressions were placed on a scale projector and viewed under a magnification of 85.5 diameters. The annuli were counted, the lateral radius from focus to margin measured, and the distance between the last annulus and the scale edge recorded. Since the fish had not begun to grow at the time that scales were taken, the distance from last annulus to margin represents growth in the year previous to capture.

Total yearly growth was related to water temperatures occurring at a depth of 20 ft, the apparent depth at which anglers catch yellow perch in summer in South Bay. Such a depth distribution is in agreement with that of perch in other areas (Odell, 1932; Carlander, 1949; Echo, 1954; Ferguson, 1958). Temperatures were determined from bathythermograph casts made at approximately weekly intervals throughout the ice-free season. Temperatures taken at three roughly evenly spaced stations in the central axis of the outer basin of the bay and at each lift of a pound net were averaged for the period of June to October inclusive, the period during which the water was usually warmer than 10 C. Such calculated mean water temperatures obtained for each of the years 1957 through 1961 and 1963 are given in Table I.

TABLE I. Mean South Bay water temperatures at a depth of 20 ft for the period June–October.

Year	Temperature (*C*)
1957	15.5
1958	14.5
1959	15.8
1960	15.2
1961	16.0
1963	15.0

The growth–temperature relationship in South Bay was compared with perch elsewhere using the growth data for yellow perch in Lower Red Lake, Minnesota, of Pycha and Smith (1954). Since water temperatures from the Red Lakes could not be obtained, air temperatures recorded at the town of Redlake, Minnesota, were used. A single mean temperature for each year was calculated from mean monthly air temperatures from June through September.

RESULTS

Growth of South Bay perch is expressed in actual scale measurements without conversion to body length or weight from empirically determined

scale dimension–body length or weight relationships (Joeris, 1956). When mean lengths were back-calculated from scale measurements for fish of certain ages, they were consistently greater than lengths calculated simply as the means of fish of those ages. This situation suggests the presence of a bias either through net selectivity for the larger members of an age group (Latta, 1959) or through a differential mortality rate acting more heavily on the smaller fish in a year-class.

The scale measurements may be considered an index of growth of the fish. Data are presented in Coble (MS, 1965) that indicates that the number of scales in adult South Bay perch is independent of length of the fish. Hence, the common assumption, that as an adult fish grows, it does not form more scales, but instead its scales increase in size with it, is supported.

Figure 1 shows the mean radius from last annulus to scale edge in relation to temperature in the year of its formation for various age groups. These data are available in detail in Coble (MS, 1965). The relationships give evidence that total annual growth of female yellow perch in South Bay is related to temperature. Regression lines placed by eye in four of the panels illustrate the relationships of growth to temperature. Extrapolation of the lines at their lower ends indicates growth beginning at mean water temperatures of 12.5–13 C.

Table II shows data on growth of yellow perch in various years in Lower Red Lake, Minnesota, and mean air temperatures at Redlake. All except the temperatures were presented by Pycha and Smith (1954). The lengths

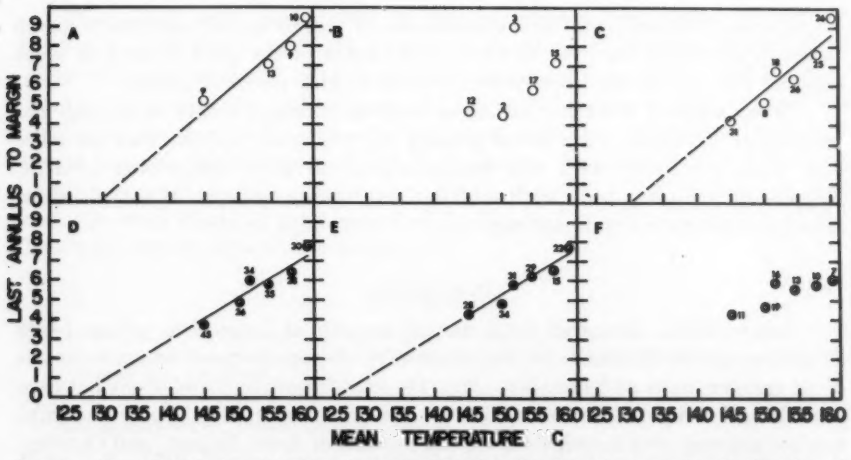

FIG. 1. Mean length (mm × 85.5) along the lateral radius from last annulus to the edge of scales of female yellow perch in relation to mean temperatures from June to October inclusive, of the water at a depth of 20 ft in the outer basin of South Bay in various years. A. Fish with VI annuli in year of capture; B. Fish with VII annuli in year of capture; C. Fish with VIII annuli in year of capture; D. Fish with IX annuli in year of capture; E. Fish with X annuli in year of capture; F. Fish with XI annuli in year of capture. Numbers beside each point represent sample sizes.

TABLE II. Lengths at first annulus, fluctuations of first-year and later growth of yellow perch in Lower Lake, Minnesota, and mean air temperatures from June to September, inclusive, at Redlake, Minnesota.

Year	No. of fish	Mean length of young (*mm*)	Percentage deviation from mean growth		Mean temperature (*C*)
			Young-of-year	Older fish	
1944	160	62.2	−0.11	12.55	16.7
1945	127	62.2	−0.11	3.12	15.6
1946	440	67.3	6.99	11.03	16.9
1947	396	61.2	−2.70	6.67	17.9
1948	136	62.0	−0.14	4.98	18.3
1949	154	62.7	−0.03	−11.09	17.6
1950	24	54.4	−13.51	−45.52	16.4
1951	44	62.2	−0.11	13.27	15.6
1952	25	55.4	−11.92	4.96	17.5
1953	110	74.6	18.60	–	17.4
1954	186	68.1	8.27	–	16.8

given were obtained from back calculations of length at first annulus of perch taken in gillnets from 1949 to 1953. The source of the growth data of older fish and the age classes represented by those figures were not given.

When each of the three measures of growth, mean length of young, and percentage deviation from mean growth for young-of-the-year and for older fish, were compared with the temperatures, no relationship was evident. Regression analyses, both with and without taking account of sample sizes, failed to indicate a significant regression (F-test; 95% level).

DISCUSSION

Jobes (1952) compared total annual growth of Lake Erie yellow perch as percentage deviations from the mean with deviations from monthly means of air temperatures at Sandusky, Ohio. He found positive correlations between growth and temperature deviations for the months of May, July, and September and negative correlations for the months of June, August, and October. If the data were combined for periods that might be considered a growing season, such as May–October or June–September, no relationship between growth and temperature was evident. Le Cren (1958), working with European perch, found a correlation between total annual growth and thermal sums over 14 C of the surface water in Lake Windermere. Hence, the work of Jobes

(1952) and the analysis here of published growth data of Pycha and Smith (1954) failed to demonstrate a relationship between total yearly growth and temperature in yellow perch. Yet the results of Le Cren (1958) for European perch and those presented here for yellow perch in South Bay indicate a strong growth–temperature relationship.

A working hypothesis might be that the growth of fish is dependent on temperature, and that other factors operate on top of that relationship. Other factors may mask the growth–temperature relationship to such an extent that it is not apparent. If such a hypothesis be correct, then it is likely that a relationship between growth and temperature would be evident in a species in one place and not in another. Differences in results obtained in the different studies, however, may be caused by differences in methodology. In the two studies in which no growth–temperature relationship was found, definite attempts were made to find one, and in both cases air temperatures were used. In the two studies in which a growth–temperature relationship was demonstrated, water temperatures were used. Differences in ages of the fish too may be important. Most of the data of Pycha and Smith concerned young-of-the-year, and the growth of fish in their first year does not always coincide with that of older fish in the same year (Pycha and Smith, 1954; Hile, 1941).

Because the data in these four studies of a growth–temperature relationship in perch were not entirely comparable, it is not possible to determine the reasons for the different results obtained.

ACKNOWLEDGMENTS

This work was carried out at the suggestion and with the aid of Dr F.E.J. Fry. Perch in South Bay were caught and sampled by the staff of the Fisheries Research Station of the Ontario Dept. of Lands and Forests. Several members of the staff, particularly Mr Daniel Cucin, were most generous with their assistance. Air temperatures from Redlake, Minnesota, were obtained through the kindness of Mr W.J. Hodge, National Weather Records Center, Federal Building, Asheville, North Carolina. The manuscript was critically read by Mr Steve Kerr and by Mr W.J. Christie, Director of the Glenora Fisheries Research Station.

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Decomposition of Cod Livers and the Formation of Free Fatty Acids in Cod Oil[1]

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ABSTRACT

The seasonal variation in the oil content of cod livers is shown to affect the liberation of free fatty acids (FFA) when the "sun-rotted" method for producing cod-oil is used under laboratory conditions. Since lean livers produce an oil with a high FFA content, oil of this type would most likely be produced in April–June after the fish have spawned. An antibiotic, chlortetracycline, was found to have little effect on the liberation of FFA and cannot be used as a liver preservative without an enzyme inhibitor added. Isopropanol at suitable concentrations inhibits both the liberation of oil from the livers and the formation of FFA in the oil.

INTRODUCTION

Cod oil, the dark-colored and usually foul-smelling oil produced by decomposing livers in barrels, still has a limited but steady market. Its principal use is in the leather tanning industry following sulfonation, and now commands as good a price as first quality, low acid, marine fats normally used in margarine. In connection with seasonal variation studies of the fatty acid composition of cod liver oil and cod flesh, a limited number of experiments were carried out to determine the effect of season, fatness, and additives on the liberation of free fatty acids during production of cod oil under laboratory conditions.

It has long been recognized that the decomposition of cod livers leading to liberation of oil, and the simultaneous splitting of the triglycerides to produce free fatty acids, is a combination of bacterial and enzymatic action. Ackman et al. (1966) have recently shown that the lipolytic enzymes in livers will produce oils with FFA as high as 23% in livers completely cold-sterilized with gamma rays. However, it appears that bacterial action is necessary to break down the liver tissues to liberate the oil.

Drummond and Hilditch (1930) pointed out the importance of steam-rendering livers within 24 hr to produce a top quality, low acid, medicinal cod liver oil. Their studies indicated that livers kept without preservatives or refrigeration gave an oil with an FFA of 0.45% after 1 day, 0.74% after 2 days, and 2.20% after 3 days. If the livers were stored at temperatures below 5 C the FFA formation was somewhat slower, i.e. 0.07% after 5 hr storage, 0.18% after 24 hr, 1.05% after 72 hr, and 1.50% FFA after 96 hr.

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They recommended processing the livers by steaming for at least 5 min to inactivate the enzymes and found that the rendered oil did not develop FFA on storage even if pieces of liver tissue or water were present. On addition of raw liver tissue, the FFA increased in stored oils. However, since clear, cold-pressed oil will not develop FFA in normal, cool storage, steaming of the livers is not necessary if water and liver tissue are removed.

Various treatments and preservatives have been proposed through the years to keep livers until it is convenient to render them. Freezing and storing at −15 to −20 C will stop bacterial and enzymatic action, and some of the oil is liberated on thawing and can be separated without steaming (Ludorff, 1953; Thorbjarnarson and Jonsson, 1946). Although Drummond and Hilditch (1930) found salt ineffective as a preservative for whole livers, this method was extensively used during the last war (Butler, 1948); however, the livers were cut to ensure thorough contact with the salt. Formalin has been employed (Butler, 1948; Brocklesby and Green, 1938), but it coagulates the water soluble proteins and hardens the livers. Brocklesby and Green (1934) showed that a *p*H lower than 5 will inhibit the fat-splitting enzymes, and proposed on this basis a method whereby minced liver brought to a *p*H of 1.5 with HCl is digested with pepsin to liberate the oil. Guttmann and Vandenheuvel (1958) proposed the use of a bacteriostat (Parasept)[2] in a solvent (isopropanol) as a liver preservative.

Johnston (1941) performed an experiment with rotting of livers in the laboratory, and found that the FFA of the extracted oil increased to 5.9% in 3 days and to 12.2% in 8 days.

EXPERIMENTAL

Samples of livers were secured each month from codfish brought in live from Terence Bay, Nova Scotia. The livers were either cut in small pieces or macerated in a Waring Blendor. The "rotting" was carried out in standard 4-oz oil sample bottles with cork stoppers and placed in a fume hood where the ambient temperature ranged from 22 to 30 C depending on the time of the year. Since the temperature during the production of commercial cod oil varies with the ambient temperature, no attempts were made to carry out the experiments under closely controlled conditions. Preliminary experiments indicated that the formation of FFA started to level off after 3–4 days, so most runs were terminated after 6–8 days. The condition or fatness of the livers was determined, according to Graham (1923), as: $\frac{100 \times \text{W of liver (g)}}{(\text{length of fish, cm})^3}$. The oil was extracted with ethyl ether after the addition of sodium sulfate, and the FFA determined according to the American Oil Chemists' Society Official Methods.

[2]Registered trade name, Heyden Chemical Corporation, New York.

The chlortetracycline (Acronize)[3] was dissolved in 100 ml water in concentrations of 10, 50, and 100 ppm. This was added to 700-g samples of homogenized livers and agitated for 10 days at room temperature after an initial thorough mixing.

RESULTS AND DISCUSSION

In Table I the results of the rotting of untreated livers are tabulated and in Table II the effects of various additives are listed.

TABLE I. Seasonal variation of FFA formation in cod oil.

Date	Number of livers	Sex	Condition of livers: 100 × wt liver / (length, cm)³	Comments	Treatment	No. of days rotted	Free fatty acids, %
Jan. 1963	2	M and F	0.022 avg	Med.	Cut	6	7.3
"	1	F	0.046	Fat	"	6	2.4
Feb. 1963	1	M	0.037	Fat	"	5	5.9
"	1	F	0.026	Med.	"	5	13.5
March 1963	1	F	0.011	Lean	"	7	25.6
April 1963	2	F	0.024	Med.	"	7	10.1
May 1963	1	F	0.017	Lean	"	7	13.0
June 1963	1	F	0.022	Med.	"	7	3.8
July 1963	1	M	0.013	Lean	"	8	23.1
"	1	F	0.045	Fat	Homog.	8	4.2
Aug. 1963	1	F	0.052	Fat	Cut	7	7.8
Sept. 1963	1	F	0.028	Med.	"	7	5.0
Oct. 1963	1	M	0.050	Fat	"	8	2.2
Nov. 1963	1	M	0.073	Very fat	"	7	2.2

The index of liver condition parallels closely other more comprehensive studies of cod from the same area in that the fat content reaches a maximum in late fall and early winter and a minimum in April–June just after spawning. The formation of FFA in the liberated oil also varies seasonally and may be correlated with the index. When the livers are lean in spring and early summer, the FFA is very high, and conversely, the oil produced from the fat livers in October–December should normally have a comparatively low FFA.

[3]Registered trade name, American Cyanamid Co.

TABLE II. Effect of various treatments on FFA formation in cod oil.

Number of livers	Sex	Condition of livers $\frac{100 \times wt}{L^3}$	Treatment	Additives	No. of days	FFA	Remarks
5 immature	F and M	0.021 avg	Homog.	21% by wt isopropanol	7	1.9	
"	"	"	"	Control	"	15.0	
1	M	0.050	Homog.	14% by wt isopropanol	8	0.5	
"	"	"	"	7% by wt isopropanol	"	0.5	
"	"	"	"	1.6% by wt isopropanol	"	1.2	
"	"	"	"	Control	"	2.2	
Several	–	–	Homog.	100 ml H_2O	10	5.0	Added to 700 g liver homogenate agitated
			"	100 ml + 10 ppm CTC	"	5.6	"
			"	100 ml + 50 ppm CTC	"	5.2	"
			"	100 ml + 100 ppm CTC	"	4.1	"
Several	–	–	Homog.	100 ml H_2O	10	6.0	Added to 700 g liver homogenate, not agitated after initial mixing
			"	100 ml H_2O + 10 ppm CTC	"	4.5	"
			"	100 ml H_2O + 50 ppm CTC	"	7.0	"
			"	100 ml H_2O + 100 ppm CTC	"	6.5	"

When very fat livers are "rotted," a large amount of oil is liberated within 2–3 days and this oil is often clean and free of water and liver tissue. The enzymes chiefly responsible for the formation of FFA are associated with the water-liver tissue phase and in the lean livers where little oil is liberated, the liberation of FFA therefore continues for the whole rotting period.

Ackman et al. (1966) have shown that the FFA formation still proceeds as rapidly in samples completely sterilized by ionizing radiation as in the controls. However, very little oil was liberated from the livers in these experiments, indicating that bacterial degradation of the liver tissues is necessary for the separation of the oil from the liver residue. The same effect was observed in the series of experiments involving the addition of isopropanol. After 7 days the oil layer in the control bottle constituted ca. 75% of the total with digested liver tissue suspended near the top, the sample with 1.6, 7, and 14% by wt isopropanol, respectively, had about 30, 20, and 10% oil on top. Since isopropanol also inhibited the FFA formation, it must affect both the lipolytic enzymes and the bacterial growth.

To determine the preservative action of antibiotics on fresh livers, various amounts of chlortetracycline in water were added to homogenized liver tissue. In no instance did the antibiotic inhibit the FFA formation of the oil even in concentrations as high as 15 ppm (100 ppm in 100 ml H_2O added to 700 g liver). This antibiotic at these concentrations would therefore be of no value as a liver preservative if used alone, again pointing out the need for an enzyme inhibitor in any liver preservative.

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Rancidity in Lean Fish Muscle. V. The Effect of Amino Acids[1]

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ABSTRACT

Addition of free amino acids to blended cod muscle affected the subsequent development of rancidity. In the presence of trace amounts of Cu^{++}, the aromatic, heterocyclic, and sulphur-containing amino acids exerted varying degrees of antioxidant acitivity. In the absence of added metallic ions the aliphatic amino acids and cysteine showed strong pro-oxidant activity. Tests were carried out to determine the effect of *p*H and the concentration of amino acid on these reactions.

Amino acid-induced rancidity was inhibited by the commercial antioxidants NDGA, PG, and BHT and by the chelating agent, EDTA; but not by tocopherol; ascorbic acid enhanced the oxidation. A limited number of tests indicated that the fish muscle did not undergo a seasonal variation in sensitivity to the amino acid-induced rancidity, and thus differed from the Cu^{++}-induced rancidity.

Those amino acids which inhibited metal-induced rancidities did not retard rancidity induced by the addition of sodium chloride.

INTRODUCTION

Free amino acids are among the numerous compounds able to change the rate at which oxidative rancidity develops in fatty foods. Lea (1936, 1938) pointed out that glycine, asparagine, and aspartic and glutamic acids are strong antioxidants. Many workers since then have investigated the effect of amino acids on oxidation of unsaturated lipids in foods and in model systems (Chang and Linn, 1964; Janicki and Gogolewski, 1958; Janneson and Marcuse, 1957; Kwapniewski et al., 1962; Lea, 1944; Marcuse, 1960, 1961, 1962; Olcott, 1958; Olcott and Kuta, 1959; Pokorory et al., 1964; Rakshit et al., 1957; Saunders et al., 1962; Scarborough and Watts, 1949; Tappel et al., 1961; Tarr, 1947; Wedemyer and Dollar, 1963). These investigations have shown that many of the amino acids have antioxidant activity. The results are not always clear cut. Cysteine, for example, has been found more often to be pro-oxidant than antioxidant; certain others have been reported as being pro-oxidant in one experiment and antioxidant in another. More recent observations (Marcuse, 1962) have shown that the same amino acid may be either pro-oxidant or antioxidant, depending upon the conditions under which reaction is taking place. Among other things, these conditions include the nature of the fatty substrate, *p*H, concentration of the amino acid, and the presence of

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phosphates, certain other salts and emulsifying agents. These findings are not unexpected considering the amphoteric nature of the amino acids, the different complexes they form with heavy metal catalysts, and the ability of certain amino acids to oxidize unsaturated lipids. Kirson and Barsily (1957), for example, have pointed out that amino acids with an amine group α to the carbonyl, give chelated complexes with Cu^{++} which do not decompose peroxides; while those with an amine group β to the carbonyl group form unchelated complexes with Cu^{++} characterized by their strong catalytic action on the decomposition of peroxides. It has also been shown that, depending upon the conditions of the experiment, an amino acid may act either as a synergist or as a primary antioxidant (Marcuse, 1960).

Most of the experimental work mentioned above was concerned with fatty foods or model systems in which the lipid phase predominated, or where lipid emulsions were finely dispersed in an aqueous solution. A somewhat different problem is present in the case of muscle of nonfatty fish. Here the fat, mostly phospholipids, is finely dispersed in the cells and bound in some manner to the muscle protein (Lovern, 1953). Although these lipids contain highly unsaturated fatty acids, because of their dispersion in the muscle, they may not be as available as free fat, either to the added metal catalysts or to atmospheric oxygen. Unlike the model systems set up for study, the muscle contains many biologically active compounds that either enter into oxidation reactions or combine with some of the products. The problem of determining the effect of free amino acids on the development of rancidity in muscle tissue is more complex than determining its effect on the oxidation of pure fatty acids or free fats or oils.

The purpose of the work described in this paper has been to determine the effect that added free amino acids have on the development of rancidity in blended cod muscle, and to do this in such a way as to take into consideration the factors of concentration of amino acid, *p*H, the effect of added copper ions, commercial antioxidants, chelating agents, and sodium chloride.

It is realized that the substrate used in these experiments, the fish muscle, is both complex and subject to seasonal and other variations. If the principal purpose had been a fundamental study of lipid oxidation, a simpler and more satisfactory substrate could have been selected. But as the object has been to study the factors affecting the development of rancidity in the muscle, per se, these inherent difficulties must be recognized and accepted.

EXPERIMENTAL

The amino acids were obtained from Nutritional Biochemicals Corp., Cleveland, Ohio, and also from Distillation Products Industries, a division of Eastman Kodak Co., Rochester, N.Y. The fish muscle was obtained from fresh cod (*Gadus morhua*), trawler-caught and less than 3 days on ice. TBA values were used as a measure of rancidity. They were made directly on the blended muscle without prior extraction, according to the method described by Tarladgis

et al. (1960). "TBA Value" indicates optical density reading at 538 μM after reaction with the thiobarbituric acid reagent.

Methods used for preparing and storing blended fish muscle were given previously (MacLean and Castell, 1964). In brief, whole fillets were blended with twice their weight of water and 75-g amounts weighed in Erlenmeyer flasks. Twenty-five grams of the solution containing the amino acids or other reagents were stirred into the blend and immediately placed at 0 C. Where *p*H adjustments were made, it was done by adding either lactic acid or sodium hydroxide solutions to the blend and then waiting until a reasonably constant reading was obtained on the *p*H meter. The recorded concentrations of materials added to the blended fish is always given in relation to the weight of the blend and not to the fish muscle itself.

During the short storage periods used in these studies (24–72 hr at 0 C), no detectable rancidity developed in the control samples of blended fish muscle. The addition of amino acids under these conditions will demonstrate those that accelerate rancidity, but will not indicate those that have an antioxidant action. For this reason, the first series of experiments was carried out only to determine which amino acids accelerated rancidity under these conditions. This was followed by tests to show the effect of amino acid concentrations, *p*H, chelating agents, and sodium chloride on some of these amino acid-induced rancidities.

The antioxidant activity of these same amino acids was determined by measuring how much they inhibited the development of Cu^{++}-induced rancidity in the muscle. This was done by adding 20 ppm Cu^{++} to the blend immediately before adding the amino acids and then following the subsequent development of rancidity in storage at 0 C. Experiments were carried out to determine the effect of concentration of amino acid, *p*H, and other factors on the inhibition of Cu^{++}-induced rancidity by amino acids showing antioxidant activity.

EXPERIMENTAL RESULTS

Pro-Oxidant Activity of Amino Acids

When added to give 0.025 M concentrations, nine amino acids accelerated rancidity in the fish muscle and 23 others caused little or no change during a 72-hr holding period (Table I). Similar tests were made after holding periods of 24, 48, 72, and 96 hr. The TBA values for the samples containing the nine amino acids listed at the top of Table I increased with increasing storage time, while the values for those in the lower portion of the Table remained almost constant and approximately the same as the controls.

In addition to the complete set of tests, made with the same fish and carried out under identical conditions, a number of other tests were made with individual amino acids at different periods of the year and using different samples of fish muscle. Results of these additional tests have been included in the right-hand column of Table I to indicate that the values are of a similar order. Tests were also made to determine whether any of the pro-oxidant amino acids would react directly with the TBA reagent to give a positive reaction. It is conceivable

TABLE I. The effect of 0.025 M concentrations of amino acids on the development of rancidity in blended cod muscle after 72 hr at 0 C.

Amino acid	*p*H	TBA	TBA[a]
L glutamine	6.7	0.340	0.130, 0.340
L lysine monohydrochloride	6.7	0.340	0.320
L (+) glutamic acid	5.2	0.330	0.220, 0.250
DL homocysteine	6.7	0.355	0.315, 0.430
L (−) leucine	6.7	0.235	0.165, 0.180
DL asparagine	6.7	0.220	0.195, 0.170
L cysteine	6.7	0.190	0.225, 0.345
L (+) arginine monohydrochloride	6.7	0.180	0.155, 0.190
DL histidine monohydrochloride	6.2	0.150	
L cystine			
DL leucine	6.7	0.020	0.020
DL alpha alanine	6.7	0.025	
beta alanine	6.7	0.015	
DL tyrosine	6.7	0.025	0.030
L tyrosine	6.7	0.015	0.020
DL isoleucine	6.7	0.015	0.020
L isoleucine	6.7	0.020	0.025
L asparagine	6.7	0.020	0.035
DL methionine	6.7	0.025	
L methionine	6.7	0.050	
DL glutamine	6.7	0.020	0.015
L histidine monohydrochloride	6.2	0.075	
hydroxy-L-proline	6.7	0.040	0.045
DL norleucine	6.7	0.045	0.020
DL aspartic acid	5.0	0.035	0.025
glycine	6.7	0.020	0.090
DL lysine monohydrochloride	6.7	0.020	0.020, 0.020
L proline	6.7	0.010	0.030
putrescine dihydrochloride	6.5	0.020	0.015
DL threonine	6.7	0.015	0.025
L arginine	8.2	0.010	0.010
DL tryptophane	6.7	0.010	0.015, 0.015
DL phenylalanine	6.7	0.015	0.020, 0.020
Control — nothing added	6.7	0.020	0.020, 0.025

[a]Additional data from other experiments carried out with different fish samples.

that they might contain traces of a reactive compound, or they may themselves decompose to form reactive compounds during distillation from the strong acid solution. The results of these tests were all negative.

Except for cysteine and homocysteine, the amino acids that accelerated rancidity were all aliphatic compounds, although all the aliphatic amino acids did not show this activity. The L and DL forms of the same amino acids did not always give the same results. The L forms of glutamine, leucine, and lysine were pro-oxidant while the DL forms had no effect. With asparagine, the DL was pro-oxidant and the L form had no effect. Neither the α nor β form of alanine accelerated rancidity.

EFFECT OF *p*H

Before adding the amino acids, some preliminary tests were made to determine the effect of different *p*H levels on the development of rancidity in

the blended muscle. Generally, decreasing the *p*H caused an acceleration in rancidity. This is shown in Fig. 1 for muscle adjusted with lactic acid and sodium hydroxide. As might be expected, it was also found that the characteristics of the acid were of more significance than the *p*H, per se, in determining the effect on the rate of oxidation. At the same *p*H level, the strongly oxidizing perchloric acid produced a high TBA value, while the chelating, tannic, and citric acids, and ascorbic acid brought about no significant change (Table II). The same applies to the use of buffers; the characteristics of the buffer as well as the *p*H, determined the effect on the development of rancidity.

The effect of six different amino acids on the development of rancidity at *p*H 6.0–7.8 is shown in Fig. 2. Differences in *p*H had the most effect where

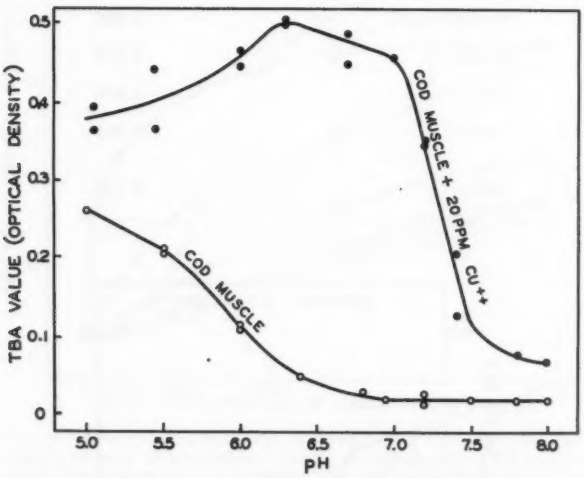

FIG. 1. Effect of *p*H on the development of rancidity in blended cod muscle with, and without, the addition of 20 ppm Cu^{++}. Initial *p*H of the muscle was 6.8; adjustments were made by adding either lactic acid or sodium hydroxide.

the two sulphur-containing amino acids were added. With cysteine, decreasing the *p*H below 7.0 resulted in greatly accelerated rancidity; with cystine the rancidity increased as the *p*H was raised in the alkaline range. With most of the other amino acids tested the tendency was for slightly increased rancidity as the *p*H was lowered. The chief difference for the "pro-oxidant" amino acids (L-leucine and L glutamic acid, i.e. Fig. 2) was that the TBA–*p*H curves were at a higher level than those which did not show this activity (DL tryptophane and L asparagine). These results confirm the observation that, generally, increased acidity favors rancidity, but the *p*H effect differs with different systems and, as in the case of cystine, the exact opposite may be so.

TABLE II. The effect on the TBA values, after 24-hr storage at 0 C, of changing the initial *p*H of blended cod muscle from 6.9 to 6.0 by the addition of nine different acids.

Acid added	TBA value
Perchloric	0.230
Aconitic	0.205
Sorbic	0.175
Trichloracetic	0.140
Benzoic	0.090
Hydrochloric	0.080
Ascorbic	0.025
Citric	0.020
Tannic	0.015
Control	0.025

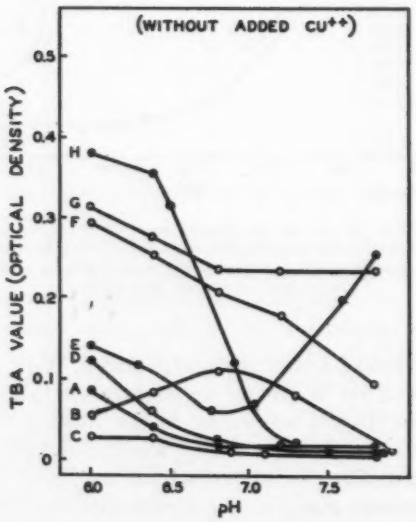

FIG. 2. Effect of *p*H on the pro-oxidant activity of amino acids in blended cod muscle as indicated by TBA values. (A) — control; (B) — DL aspartic acid; (C) — DL tryptophane; (D) — L asparagine; (E) — L cystine; (F) — L leucine; (G) — L glutamic acid; (H) — L cysteine.

Effect of Amino Acid Concentration

The extent to which the concentration of the pro-oxidant amino acids could be reduced and still accelerate rancidity differed with different amino acids. A 0.025 M concentration was selected for most experiments because it produced consistently measurable changes within the 72-hr storage period. With some amino acids the pro-oxidant activity was still evident at 0.05 and 0.002 M concentrations (Fig. 3).

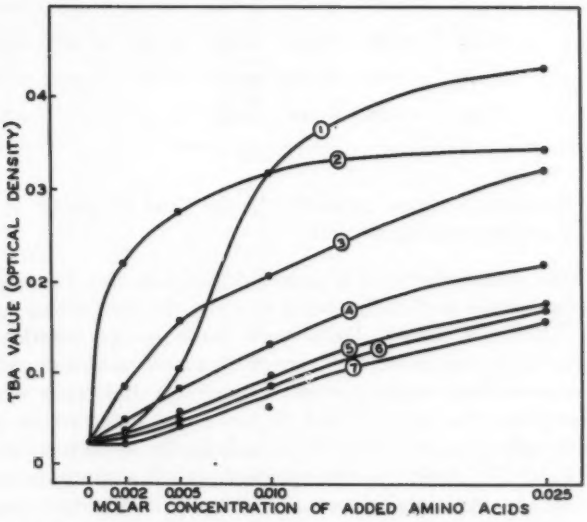

FIG. 3. Effect of concentration of seven amino acids on their pro-oxidant activity in blended cod muscle as indicated by TBA values. (1) DL homocysteine; (2) L cysteine; (3) L lysine monohydrochloride; (4) L leucine; (5) DL asparagine; (6) L glutamine; (7) L arginine monohydrochloride.

Antioxidants and Chelating Agents

The pro-oxidant activity of amino acids can be retarded by many of the reagents normally used to inhibit lipid oxidation (Table III). The commercial antioxidants, propyl gallate (PG) and nordehydroguaiaretic acid (NDGA) strongly inhibited the reaction; butylated hydroxytoluene (BHT) was not quite so effective. The chelating agent ethylenediamine tetraacetic acid (EDTA) also strongly inhibited this reaction. Citric acid and alpha-tocopherol had little or no retarding action, and ascorbic acid apparently acted as a synergist, further accelerating the oxidation. In another set of tests, 0.01% of EDTA completely inhibited the pro-oxidant action of 0.025 M concentrations of DL homocysteine, DL lysine monohydrochloride, L glutamine, and L glutamic acid. The addition of 0.1% alpha-tocopherol had no effect.

TABLE III. Effect of antioxidants and chelating agents on amino acid-induced rancidity in blended cod muscle.

Amino acid (0.025 M concentration)	TBA values after 24 hr at 0 C							
	Untreated controls	0.1% alpha tocopherol	0.05% ascorbic acid	0.05% citric acid	0.01% BHT	0.01% PG	0.01% NDGA	0.01% EDTA
DL asparagine	0.195	0.180	0.315	0.140	0.075	0.020	0.020	0.200
L (–) leucine	0.180	0.190	0.340	0.180	0.080	0.020	0.025	0.015
L cysteine	0.135	0.125	omitted	omitted	0.030	0.015	0.015	0.015
None added	0.020	0.020	0.040	0.020	0.020	0.015	0.020	0.015

SEASONAL VARIATIONS IN THE SENSITIVITY OF COD MUSCLE TO AMINO ACID-INDUCED RANCIDITY

It had been found that cod muscle is more sensitive to copper-induced rancidity in the winter and early spring than in the late spring and summer (Castell and MacLean, 1964). Tests were made to see whether the same variation occurred in the muscle in regard to amino acid-induced rancidity. The muscle samples from eight summer-caught fish and eight winter-caught fish were divided into two portions and 20 ppm Cu^{++} added to one portion and 0.04M L cysteine added to the other. The figures for the subsequent development of rancidity (Table IV) show that the muscle from the summer-caught fish was not resistant to the cysteine-induced rancidity as it was to copper. Similar results were obtained with L glutamine and glutathione.

ODOURS ACCOMPANYING AMINO ACID-INDUCED RANCIDITIES

The odours accompanying amino acid-induced rancidities in the muscle are not those normally associated with the term "rancidity." The strong "oily," "rancid fish oil," or "paint-like" odours were never encountered. But very definite "off" odours did develop and they were different with different amino acids.

Some amino acids, particularly those containing sulphur, have characteristic odours to begin with, but these changed and grew stronger after being added to the muscle. With cysteine a very strong hydrogen sulphide-like odour developed which later became "seaweedy," similar to odours characteristic to the early stages of Cu^{++}-induced rancidity. Homocysteine developed odours suggesting mixtures of onion pickles and hydrogen sulphide. The odours from the nonsulphur-containing, pro-oxidant amino acids were less obnoxious and difficult to describe. They were generally recorded as being "off," "fish-off," or "strong off-odour." These odours coincided with increased TBA values ranging

TABLE IV. The effect of season on the development of Cu^{++}-induced and cysteine-induced rancidity in blended cod muscle. In each case the tests were made on muscle prepared from the same fillet.

Date of catching	TBA values (0.04 M)	
	With added 20 ppm Cu^{++}	With added 0.5% cysteine
November 13	0.320	0.245
" 13	0.640	0.280
" 19	0.400	0.330
" 24	0.540	0.245
" 25	0.320	0.105
" 25	0.580	0.200
December 8	0.220	0.240
January 19	0.535	0.135
Average:	0.444	0.222
June 17	0.062	0.310
" 17	0.055	0.115
" 25	0.025	0.120
July 2	0.045	0.320
" 12	0.137	0.245
" 17	0.070	0.312
" 18	0.035	0.195
" 20	0.020	0.115
Average:	0.056	0.218

between 0.180 and 0.380. They did not develop following the addition of any of the amino acids shown in the lower portion of Table I.

Different *p*H values, even with the relatively narrow range of 5.5–7.5, frequently had a profound effect on some of the types of odours that were produced. Cysteine, for example, with fish at *p*H 6.6–7.0 produced a strong odour of cooked egg yolk. When the *p*H was reduced to 5.8–6.3, sour onion and mercaptan-like odours became predominant.

ANTIOXIDANT ACTIVITY OF AMINO ACIDS FOR COPPER-INDUCED RANCIDITY

Many amino acids showed some capacity to retard copper-induced rancidity in the muscle, and some were quite strong antioxidants (Table V). The aromatic, heterocyclic, and sulphur-containing compounds showed the strongest activity, while the aliphatic compounds had little or none. In the Table, arginine appears to be an exception, but this strongly basic compound raised the *p*H to a level that retards oxidation; when used in the form of a monohydrochloride this apparent antioxidant activity completely disappeared. The inhibiting action of 0.04 M concentrations of eight amino acids and glutathione, in the presence of 0–30 ppm added Cu^{++}, is shown in Fig. 4. Even with 30 ppm Cu^{++}, rancidity did not develop where L cysteine and DL aspartic acid had been added. L cystine and DL tryptophane suppressed rancidity in the presence of

TABLE V. The inhibiting effect of 0.025 M concentrations of amino acids on the development of copper-induced rancidity in blended cod muscle during a 24-hr storage period at 0 C.

Amino acid	Type	Odour	*p*H	TBA	TBA[a]
glycine	aliphatic	strong seaweedy	6.7	0.465	0.205
DL glutamine	"	"	6.7	0.460	0.205
DL theonine	"	"	6.7	0.445	0.380
DL asparagine	"	seaweedy	6.7	0.430	0.270, 0.345
L lysine mono-HCl	"	strong seaweedy	6.7	0.385	
L glutamic acid	"	seaweedy	5.1	0.380	0.335, 0.295
L leucine	"	"	6.7	0.380	0.235
DL isoleucine	"	"	6.7	0.380	0.140
DL valine	"	strong seaweedy	6.7	0.360	0.230
L asparagine	"	"	6.7	0.360	
L arginine mono-HCl	"	seaweedy	6.7	0.355	0.180
DL alanine	"	"	6.7	0.300	0.100
L glutamine	"	"	6.7	0.220	
DL lysine mono-HCl	"	"	6.5	0.205	0.310
hydroxy-L-proline	heterocyclic	slight seaweedy	6.7	0.200	0.105, 0.085
DL histidine mono-HCl	"	NMO	6.0	0.170	0.085
DL norleucine		slight seaweedy	6.7	0.170	0.160
L proline	"	slight seaweedy (?)	6.7	0.165	0.105
L cystine		"	6.7	0.115	0.095, 0.085
L cysteine		hydrogen sulphide	6.7	0.095	0.020, 0.025
putrescine di-HCl		NMO — musty (?)	6.6	0.095	0.015
DL phenylalanine	aromatic	NMO	6.7	0.065	0.015
DL aspartic acid		sour-off	5.0	0.065	0.050
DL tyrosine	aromatic	NMO	6.7	0.065	0.030, 0.050
L tyrosine	"	"	6.7	0.060	
DL tryptophane	heterocyclic	"	6.7	0.035	0.025, 0.020
DL arginine	aliphatic	paste-like	8.2	0.055	0.010, 0.025
Glutathione	tripeptide	mercaptan-like		0.270	
Taurine	nitrogen base				
Creatine	amine				
Betaine HCl	nitrogen base	NMO	5.1		
Control with Cu^{++}			6.7	0.310	
Control without Cu^{++}			6.7	0.030	

[a]Additional data from other experiments carried out with different fish samples.

20, but not 30 ppm Cu^{++}. L glutamic acid and L histidine were a little less effective. The results with DL methionine were indistinguishable from the controls containing no added amino acids. The more acidic glutathione and cysteine hydrochloride were strongly pro-oxidant in the absence of copper and showed little or no change with the addition of up to 30 ppm Cu^{++}.

*p*H AND ANTIOXIDANT ACTIVITY OF AMINO ACIDS

Differences in *p*H affected the development of rancidity in fish to which only Cu^{++} had been added (Fig. 1). The rate was greatest in the range of *p*H 6.3–7.0. Increasing alkalinity between 7.0 and 7.5 caused a marked decrease; increasing the acidity below 6.3 caused a similar but more gradual decrease. It was always observed that whenever an acid reaction resulted in denaturation of the fish muscle proteins, copper-induced rancidity was greatly reduced. This generally occurred when the *p*H was reduced below the isoelectric point of the

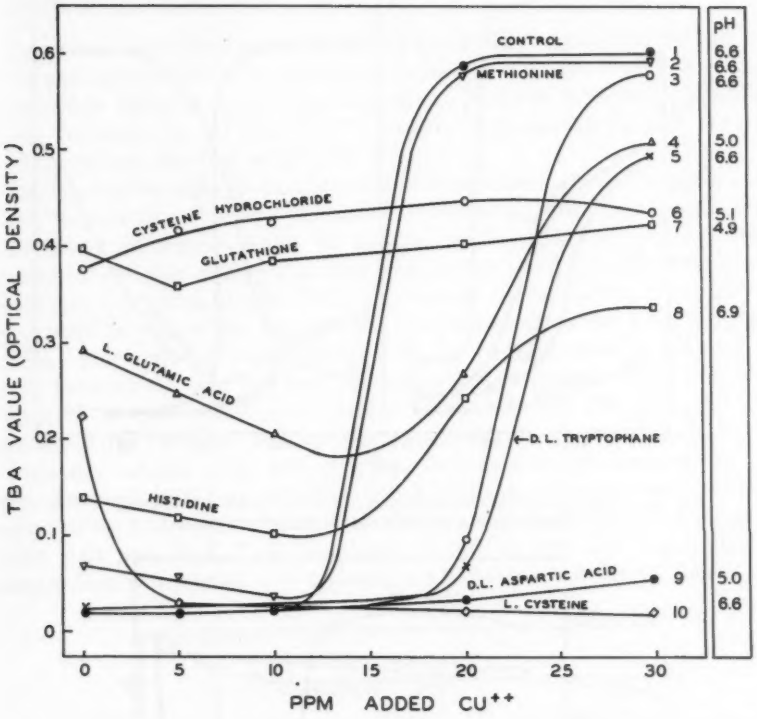

FIG. 4. Effect of adding several amino acids and glutathione on the development of rancidity in blended cod muscle to which 0, 5, 10, 20, and 30 ppm Cu^{++} had also been added. The TBA values are after 48 hr storage at 0 C. The *p*H values in the column at the right are for the samples containing 20 ppm Cu^{++}.

fish muscle proteins. According to Connell and Howgate (1964) this is approximately *p*H 5.4 for cod muscle.

The *p*H also affected the antioxidant activity of various amino acids for copper-induced rancidity. This was different for different amino acids. Figures 5A and B show the results with cysteine and cystine. When added separately both cysteine and Cu^{++} accelerate rancidity at *p*H 6.0–7.0; above 7.0 their activity is rapidly suppressed. When added together, the TBA–*p*H curve is similar but lower than that of the cysteine alone, indicating that the more abundant cysteine has completely complexed with the Cu^{++}. With cystine, below *p*H 6.5 the amino acid completely inhibited the action of the Cu^{++}. Above this point the Cu^{++} and cystine produced higher results than either of them alone. Curves showing how *p*H affects the inhibiting action of five other amino acids on Cu^{++}-induced rancidity are given in Fig. 6.

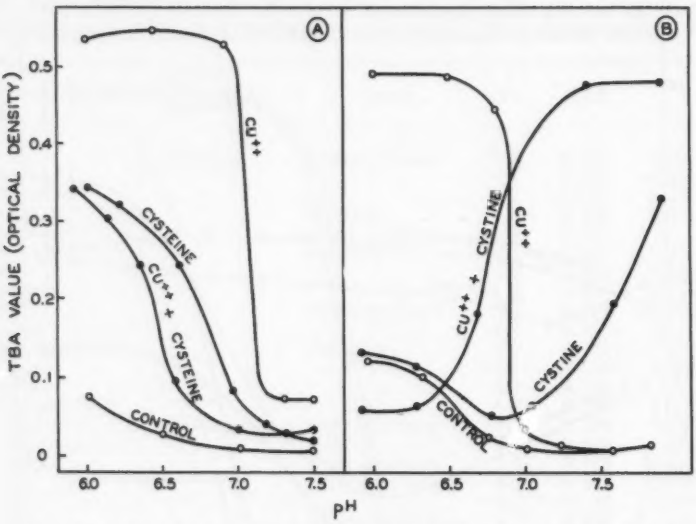

FIG. 5. Effect of *p*H on the inhibition of Cu^{++}-induced rancidity by 0.04 M concentrations of (A) cysteine and (B) cystine.

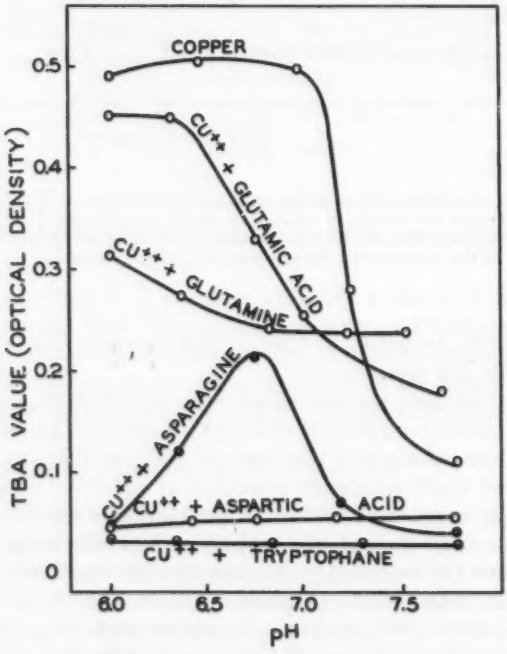

FIG. 6. Effect of *p*H on the inhibition of Cu^{++}-induced rancidity by 0.04 M concentrations of five amino acids.

Effect of Amino Acid Concentration on Inhibition of Cu^{++}-Induced Randicity

The amino acids differ in the extent to which they can be diluted and still exert an antioxidant action on the copper-induced rancidity. Tryptophane and cysteine, when added to give a concentration of 0.01% (100 ppm), inhibited rancidity produced by 20 ppm Cu^{++}; tyrosine required 0.05% and aspartic acid was even less effective with 0.1% (Fig. 7).

The relation between concentration and antioxidant activity is much more complex for cysteine than for the other, nonsulphur-containing compounds. This is shown in greater detail for cysteine in Fig. 8A and B. Very small concentrations of this amino acid were required to offset the effects of Cu^{++}. With 20 ppm Cu^{++} and 10 ppm (0.001%) cysteine the odour was changed from "oily rancid" to the milder and less offensive seaweedy odour (Fig. 8B), and the TBA value reduced from 0.48 to 0.38; 20–50 ppm cysteine lowered the TBA to between 0.1 and 0.2 and prevented all off odours. With additional increases in the concentration of cysteine between 50 and 500 ppm, the TBA values gradually increased and were again accompanied by the production of the seaweedy odours (Fig. 8A). Further increases in the concentration of cysteine between 1000 and 3000 (0.1 and 0.3%) cause the seaweedy odour to be replaced by hydrogen sulphide-like odours and reduces the TBA values to between 0.01 and 0.02. This experiment using 20 ppm Cu^{++} and varying concentrations of cysteine was repeated six times with different lots of fish

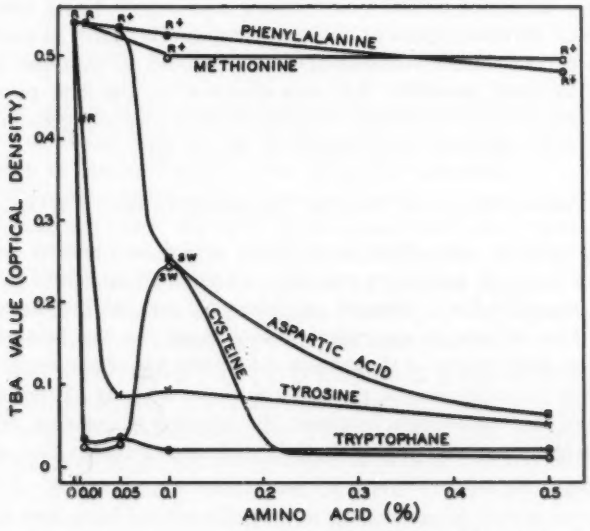

FIG. 7. Effect of concentration of six amino acids on their ability to inhibit Cu^{++}-induced rancidity as indicated by TBA values and odours. R = oily rancid odour; R+ = rancid plus other off-odours; SW = seaweedy odours.

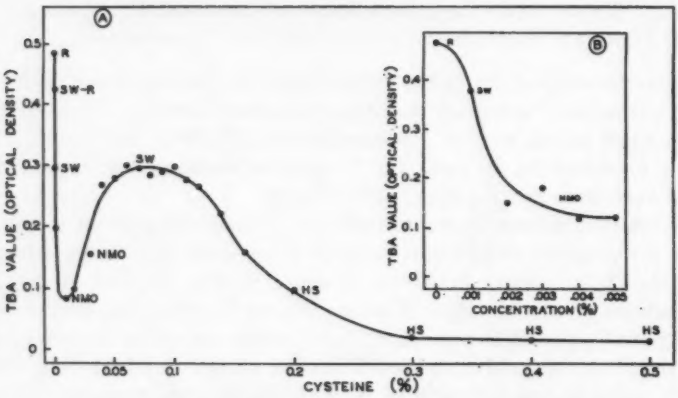

FIG. 8. Effect of concentration of added cysteine on the inhibition of Cu^{++}-induced rancidity in blended cod muscle after 24 hr at 0 C. The inset shows the results from another test with still lower concentrations of cysteine. The added Cu^{++} was 20 ppm. Odours: R = rancid; SW = seaweedy; HS = hydrogen sulphide-like odours; NMO = not much odour.

muscle. Similar shaped curves at approximately the same cysteine concentrations were obtained in each experiment but the height of the hump and the strength of the accompanying seaweedy odours showed some variation. In this area the maximum TBA values varied from 1.5 to 3.5 and the odours from "those merely described as 'off' " to "strong seaweedy." These results suggest that very small concentrations of cysteine complex the Cu^{++} in such a manner that both are neutralized. Additional concentrations of cysteine bring about the cysteine-induced rancidity that was observed in the first portion of this paper. No explanation is available at this time as to why still greater concentrations of cysteine reduce the TBA values to almost nil.

Effect of Amino Acids on Sodium Chloride-Induced Rancidity

Sodium chloride and other electrolytes added to blended muscle bring about a mild type of oxidative rancidity (Castell et al., 1965). The amino acids which retarded Cu^{++}-induced rancidity did not inhibit the salt-induced rancidities. The picture is somewhat complicated by the fact that, in the concentrations used, many of these salts reduce the *p*H of the muscle; and this, in turn, affects the oxidation of the lipids and the activity of the amino acids. Irrespective of the mechanism, however, the addition of cysteine, tryptophane, tyrosine, methionine, or phenylalanine did not reduce the salt-induced rancidity.

Effect of Protein Hydrolysates

The mixture of amino acids in hydrolized proteins includes both those that are pro-oxidant and those that are antioxidant. Several tests were carried

out in which hydrolysates of yeast, casein, and lactalbumin were added to the blended muscle to give concentrations of 0.5%. In the absence of added Cu^{++} during a 48-hr storage period, these hydrolysates caused the TBA values of the muscle to increase from 0.02 to 0.225, 0.135 and 0.185, respectively. The hydrolysates themselves had characteristic odours to begin with, but during storage these became stronger and more offensive.

When the hydrolysates were added to muscle containing 20 ppm Cu^{++} during a 24-hr storage period they kept the TBA values down to 0.030, 0.040 and 0.060, and prevented the production of the rancid odour. The control sample with copper had a TBA value 0.475 and smelled strongly rancid.

Tests were also made to determine the extent to which the hydrolysates could be reduced in concentration and still be effective in retarding the Cu^{++}-induced rancidity. In most cases, in concentrations below 0.1% they lost their capacity to retard the copper-induced rancidity.

Inhibition of Iron-Induced Rancidity by Amino Acids

All experiments reported to this point have dealt with the inhibition of copper-induced rancidity by amino acids. Parallel experiments were carried out using iron as the catalyst. These will be discussed in detail in a subsequent paper. It was found that some of the amino acids that retarded the copper-induced rancidity also retarded rancidity catalyzed by the addition of ferrous and ferric ions. The same amino acids did not retard oxidation accelerated by the addition of either sodium chloride or hydrogen peroxide.

SUMMARY AND DISCUSSION

From the results of these experiments it is obvious that statements regarding the effect of amino acids on the development of rancidity must be qualified by a definition of the conditions under which the experiment has been carried out. Under different conditions the same amino acid may exert both antioxidant and pro-oxidant activities. The *p*H of the muscle and the presence of copper ions are two of the factors that influence this activity. This agrees with the previous work of Marcuse (1962) who found that *p*H, the nature of the substrate, and the presence or absence of phosphate could alter the action of the amino acids on the oxidation of linoleic acid and its methyl ester.

In the absence of added Cu^{++}, L glutamine, L lysine monohydrochloride, L leucine, L glutamic acid, L cysteine, DL homocysteine, and DL asparagine accelerated rancidity as indicated by TBA values. This oxidation could be retarded by the addition of the antioxidants NDGA or PG and by the chelating agent EDTA. It was almost unaffected by citric acid or α-tocopherol, and it was actually enhanced by the addition of ascorbic acid. It was shown that the positive TBA reaction did not occur as the result of contamination of the amino acid with traces of a reactive compound or as a result of by-products formed during distillation from the strongly acidified substrate. The TBA values from

these amino acid-induced rancidities never reach the high values that can be obtained by addition of trace amounts of Cu^{++} or Fe^{++}, and the typical odours usually associated with rancid fish oil did not develop. However, these oxidations were accompanied by off odours which differed with different amino acids.

A different picture was observed when individual amino acids were added to blended muscle to which traces of Cu^{++} had been added. Some strongly retarded the copper-induced rancidity. L cysteine, DL trytophane, L and DL tyrosine, aspartic acid, and DL phenylalanine were particularly effective, but others also exerted varying degrees of antioxidant activity. In general, the aliphatic compounds were poor antioxidants and the more complex aromatic and heterocyclic types were much stronger antioxidants. In addition to the low TBA values, the antioxidant effect could readily be observed by the absence of rancid or other off-odours.

The *p*H has a considerable influence over the activity of the amino acids in their relationship to rancidity. In the absence of added Cu^{++}, cystine became increasingly pro-oxidant with increasing alkalinity above *p*H 7.0. All the other amino acids tested were more active pro-oxidants as the *p*H was decreased below neutrality. Increasing acidity had the greatest effect on cysteine. Where the rancidity was accelerated by Cu^{++}, the inhibiting effect of the amino acids was also affected by *p*H and generally tended to decrease with increasing acidity. Tryptophane and aspartic acid were the least affected by *p*H. In the alkaline range cystine and Cu^{++} accelerated rancidity to a much greater extent than either Cu^{++} or cystine alone.

There is a great temptation to transpose some of these results to fish under commercial conditions and suggest which of the amino acids might be useful as commercial antioxidants. It must be remembered, however, that these antioxidant studies have been confined to copper-induced rancidity. Until similar studies have been made for rancidity accelerated by iron and other activators, one must hesitate before drawing conclusions regarding their commercial application. It must also be remembered that in addition to retarding lipid oxidation, some of these amino acids may produce very offensive odours and flavours. There are some indications that cysteine, for example, if used within a very narrow range of concentrations, might be an effective commercial antioxidant for fish; but, used a little in excess of this range and hydrogen sulphide-like odours would develop. It must also be remembered that the antioxidant activity of most amino acids increases with increasing acidity, but many of our commercial fish have *p*H values close to neutrality. With these reservations in mind, our experience suggests that aspartic acid and tryptophane show the most promise as commercial antioxidants for use with fish flesh.

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A Hematological Study on the Mountain Whitefish, *Prosopium williamsoni*[1,2]

By Ida M. McKnight[3]

ABSTRACT

A hematological study was made on 119 mountain whitefish (*Prosopium williamsoni*) from the Yellowstone and Madison River drainages in Montana, 1961–62. Hemoglobin determinations had a range of 7.0–13.7 g/100 ml (mean 10.6 g) while hematocrits had a range of 28.5–62.2% (mean 43.5%). Erythrocyte counts ranged from 1.01 to 2.34 million/mm³ (mean 1.57 million) and leucocyte numbers ranged from 360 to 25,500/mm³ (mean 5400). Thrombocyte counts ranged from 870 to 47,200/mm³ of blood (mean 17,600). Leucocyte differential counts had a mean of 33.9% lymphocytes and 66.1% neutrophilic granulocytes with the predominant granulocyte being the lobed mature form. Granulocyte means were higher in leucocyte counts over 8100/mm³. Few immature cells were seen in blood smears. Some polychromatic erythrocytes were found and myelocytes were the most immature granulocytes seen. Differences were observed in the various values between small and large fish, males and females, and for fish taken in summer and winter. The kidney was the active hemopoietic tissue, forming granulocytes, lymphocytes, and thrombocytes. The spleen produced most of the erythrocytes and occasionally other cell types. The liver was hemopoietic in some anemic fish.

INTRODUCTION

A HEMATOLOGICAL STUDY on the mountain whitefish (*Prosopium williamsoni*) was conducted for the purpose of testing various hematological techniques, determining normal values, and blood cell origins. Fish were collected from the Yellowstone and Madison River drainages, Montana, at all seasons during 1961–62.

Numerous studies on fish blood show inconsistent results possibly due to techniques as reported by Lieb et al. (1953), Larsen and Snieszko (1961), and Haws and Goodnight (1962). The only study on mountain whitefish encountered was that of Bolton (1933) who studied basophil (mast) cells. Many publications, however, concern the Salmonidae (Katz, 1951; Black, 1955; Lieb et al., 1953). Hematological investigations have also been made on other fishes: Jordon and Spiedel (1924) studied several teleosts; Yokayama (1947) the yellow perch (*Perca flavescens*); Tyler (1960) two Antarctic fishes (*Trematomus borchgevinki* and *Notothenia larsoni*); and Young (1949) the opaleye (*Girella nigricans*).

MATERIALS AND METHODS

Mountain whitefish were collected by angling and held in nets placed in the stream until transported to the laboratory. Because they were extremely sensitive to high temperatures, ice was used in the containers employed for

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transportation during warm weather. Most of the fish were brought to the laboratory before blood samples were removed, but blood was taken from some fish at the site of capture. Other fish were held in laboratory troughs for varying periods without food. Prior to taking blood samples, most fish were rapidly anesthetized with a 1:20,000 dilution of tricaine methanesulfonate (M.S. 222). Each fish was measured, weighed, and sexed, and scales were removed for age determinations. Some were kept alive following the removal of blood. These were individually tagged and held in troughs for various periods for subsequent sampling.

A laboratory test exposing fish to varying dissolved oxygen conditions during a 2-hr period and involving anesthetized and unanesthetized fish showed no effect on blood values. Because fish blood clots rapidly anticoagulants were selected which prevented clotting yet caused no hemolysis of erythrocytes. By rinsing the syringe with a 10% solution of dipotassium ethylenediamine tetraacetate (EDTA) and expelling all excess it was possible to obtain a satisfactory blood sample. The dry chemical did not dissolve readily in fish blood. Wintrobe (1962) reported on the use of various anticoagulants and recommended EDTA for human hematology. Heparin also has been used satisfactorily as an anticoagulant by many investigators but it did not prevent clotting as completely as EDTA when mixed with mountain whitefish blood.

Blood was removed from the caudal artery by the use of a syringe (needle size 22). The needle was inserted anteriorly (just posterior to the anal fin) until it struck the vertebral column at an angle of 45°. It was then retrieved about 1 mm until a steady flow of blood was obtained. Approximately 0.5 ml was taken from fish of 40–85 g and 1–2 ml from larger fish.

Hemoglobin

In order to determine the most satisfactory hemoglobin method, an experiment was performed using oxyhemoglobin, acid hematin, and cyanmethemoglobin techniques on each of six fish (Table I). Dilutions were made

Table I. Hemoglobin values (g/100 ml blood) determined by various methods.

Sample number	Cyanmet-hemoglobin	Oxyhemo-globin	Acid hematin Klett-Summerson	Acid hematin Sahli
1	11.61	11.57	14.12	13.67
2	7.52	7.67	9.90	10.13
3	8.32	8.29	10.95	10.80
4	9.76	9.52	12.33	11.70
5	8.28	8.50	10.90	10.10
6	5.65	5.88	8.65	8.40

with the same 0.02-ml pipette thus eliminating variations due to differences in pipettes. Two or three determinations were performed for each method and the mean recorded. A Klett-Summerson photoelectric colorimeter was used for all hemoglobin tests except those done with the acid hematin technique by the Sahli method. Ortho's Aculobin Hemoglobin standard was used to determine equivalent colorimetric readings and curves were then made from these results. This was found to be an accurate and practical method of calibrating the colorimeter. Cyanmethemoglobin determinations were made with dilutents prepared with Ortho's pellets. Comparisons between the use of Drabkin's solution and the Ortho dilutent showed both fluids to be satisfactory for fish blood hemoglobins. I preferred the pellets because of the ease with which solutions were prepared. Wintrobe (1962) reported that the cyanmethemoglobin method is accurate. It was used satisfactorily with fish blood by Sindermann and Mairs (1961). Because erythrocyte nuclei were not destroyed when acid hematin methods were used, these were considered unsatisfactory. The oxyhemoglobin technique gave similar results to those of cyanmethemoglobin.

Hematocrit

In most cases blood used for hematocrit determinations was taken from the same sample as that for hemoglobin tests. Blood was allowed to run into one end of a capillary tube (70 mm long, 0.5–0.9 mm ID). About three-fourths of the tube was filled, sealed with plastic clay, and spun for 7 min in a microhematocrit centrifuge at a speed of 12,500 rpm. No anticoagulant was used in capillary tubes because there was enough in the drawn blood sample. The volume of packed erythrocytes was determined with a hematocrit tube reader and reported as per cent of whole blood.

In order to determine the effects of anticoagulants, capillary tubes were rinsed with either 10% solutions of ammonium oxalate, EDTA, or heparin using the technique suggested by Larsen and Snieszko (1961). Commercially prepared heparin tubes were also used. The caudal peduncle was severed and wiped with a dry towel and freely flowing blood was drawn into the tubes. All tests were made in duplicate, the average recorded, and the amount of hemolysis noted. No more than four samples could be taken from one fish without getting clots in the capillary tubes thus permitting the testing of only two anticoagulants on a single fish. When using this method, clots were found in three of the four oxalate tubes and hemolysis was observed in two of four EDTA tubes. Clots were also found in five of eight commercial heparin tubes. No clotting occurred in laboratory-prepared heparin tubes and no hemolysis was seen in either type of heparin tubes used. Results indicate the need for great caution in the use of anticoagulants for hematocrit determinations. Ten per cent ammonium oxalate and EDTA solutions were unsatisfactory for preparing hematocrit tubes. The tubes prepared with heparin in the laboratory were superior. This was also found to be true by Larsen and

Snieszko (1961). Mairs and Kennedy (1962) used commercial heparin tubes in a study of warmwater fishes and found them satisfactory only for the bluegill (*Lepomis macrochirus*).

A comparison was made between the effect of EDTA when used in liquid and crystalline forms. After blood was taken from the caudal artery using a syringe rinsed with 10% EDTA solution, laboratory-prepared EDTA tubes were filled with blood from the cut surface. No clots or hemolysis were observed in tubes containing blood that had been treated with the liquid EDTA. Three of four tubes with crystalline EDTA gave higher results due to clots. It was concluded that liquid EDTA readily prevents clotting when used in a syringe for blood drawing but is unsatisfactory for hematocrits when used in the crystalline form probably due to insolubility.

Erythrocytes

Erythrocyte counts were made on blood drawn from the caudal artery and treated with EDTA. Rees-Ecker's (Wintrobe, 1962), Yokayama's (1947), and Hendricks' (Hesser, 1960) diluting solutions were used. Blood was diluted 1:100 in a standard Thoma red cell diluting pipette. After mixing for 1–2 min, diluted blood was flooded onto both sides of a Spencer bright-line hemocytometer. A petri dish containing damp filter paper was placed over the chamber to hinder drying and dilutions were allowed to settle several minutes before counting. Five of the 0.25-mm^2 ruled areas (depth 0.1 mm) were counted on each side of the chamber and the total erythrocyte count/mm^3 of blood was determined by the formula:

$$\frac{\text{number of cells counted} \times \frac{1}{\text{depth}} \times \frac{1}{\text{dilution}}}{\text{number mm}^2 \text{ counted}}.$$

Similar results were obtained with all three diluting fluids. Hendricks' solution was more stable and more convenient to use than the others. Rees-Ecker's and Yokayama's solutions gave more accurate counts when used with anemic blood because they stained immature erythrocytes, facilitating their separation from mature cells.

Lengths and widths (microns) of mature erythrocytes treated with Wright's stain were secured by the use of an ocular micrometer at a magnification of 1000. A total of 30 cells was taken at random in consecutive fields (1–5) on one slide for each fish and ranges and means were computed.

Leucocytes

Direct and indirect methods were used to determine the number of leucocytes/mm^3 of blood. For the direct method, blood was taken from the caudal artery, treated with EDTA, and was diluted 1:10 in a Thoma white cell diluting pipette using Yokayama's dilutent. Pipettes were shaken by hand for

1–2 min and diluted blood was flooded onto a Spencer bright-line hemocytometer. Cells were allowed to settle several minutes on the chamber which was covered by a petri dish as previously described for erythrocyte counts. In order to count only the leucocytes, it was necessary to differentiate between leucocytes and thrombocytes (Yokayama, 1947). The total ruled area (9 mm^2) of the hemocytometer was counted and the number of leucocytes/mm^3 of blood was computed as for erythrocytes.

Blood was taken from the caudal artery for the indirect method. The first drop of blood from the needle was used to make a smear which after drying was treated with Wright's stain. Blood treated with EDTA was diluted 1:100 and the total number of erythrocytes determined. The stained smear was examined under the microscope using a magnification of 1500. The number of leucocytes found per 7000 erythrocytes was recorded when counting only adjacent microscopic fields which contained no greater than 60 erythrocytes. The total number of leucocytes/mm^3 of blood was computed by the following formula:

$$\text{leucocytes/mm}^3 = \frac{\text{number of leucocytes counted} \times \text{erythrocytes/mm}^3}{7000}.$$

The same blood sample was used for direct and indirect methods. The indirect method was found to be more accurate. Because of the great similarity between leucocytes, thrombocytes, and immature erythrocytes as seen on the hemocytometer, it was not always possible to accurately differentiate between these cells. Lucas and Jamroz (1961) reported errors when using both techniques with avian blood, but recommended the indirect method.

Smears were made from blood not mixed with anticoagulants. These were allowed to dry and then treated with Wright's stain. Total leucocyte counts were determined by the indirect method and then differential counts were made from the same slide. When the total count was 2000 or less, 50 cells were differentiated on one slide for each fish and percentages of cell types were determined. When total counts were greater than 2000, 100 or more cells were differentiated.

Thrombocyte Count

The indirect method was used following the technique given for leucocyte counts. The percentage of thrombocytes was determined from a stained blood smear and the total number of cells/mm^3 of blood was calculated. These counts were not considered as accurate as leucocyte counts because of rapid thrombocyte clumping which caused poor distribution on the smear. Wintrobe (1962) stated that no technique is completely satisfactory for human thrombocyte counts and Lucas and Janroz (1961) preferred the indirect method for avian thrombocyte counts. No reports were found of thrombocyte counts on fish blood.

Erythrocyte Sedimentation Rate

Blood was taken from the caudal artery and mixed with EDTA. Sedimentation rates were determined by the use of a Landau-Adams microsedimentation apparatus (Landau, 1933). Blood was allowed to settle for 1 hr in upright supported pipettes and the upper level of packed erythrocytes was recorded in millimeters. Although this method was not very satisfactory, results showed some consistencies.

Blood Clotting Time

The caudal peduncle was severed, wiped with a dry towel, and fresh blood was allowed to flow into a capillary tube (70 mm × 0.5–0.9 mm ID). The time in seconds was recorded when the blood showed a thread-like clot between broken ends of the capillary tube.

Mature and Immature Blood Cells

Cells of mountain whitefish were studied (1500 ×) on blood smears, and on kidney, liver, and spleen imprint smears which had been treated with Wright's stain.

RESULTS

Hemoglobin

Hemoglobin ranges and means were determined on 119 mountain whitefish (ages 1–6 years) collected from April 1961 to September 1962 (Table II). The range was 7.0–13.7 g/100 ml blood with a mean of 10.6 g. Six fish taken in April 1961 had a mean of 12.8 g, however, this may have resulted from an inadequate sample. A comparison based on weights (Table III) determined for 95 fish taken at various times showed that 11 fish (40–109 g; 1–2 years) had a range of 7.6–11.2 g hemoglobin (mean 9.6 g) while 84 fish (110–650 g; 2–6 years) had a range of 7.8–13 g (mean 10.6 g). These results suggest slightly lower hemoglobin values for smaller fish. A comparison was made between males and females (Table IV). A total of 36 males taken at various times had a range of 7.9–13.0 g (mean 10.9 g) while 51 females had a range of 7.0–12.6 g (mean 10.1 g). Murachi (1959) reported a hemoglobin range of 3.3–16.9 g for young carp. Black (1955) gave the following means: 10.0 g, Kamloops trout (*Salmo gairdneri kamloops*); 9.8 g, squawfish (*Ptychocheilus oregonensis*); 8.1 g, largemouth bass (*Micropterus salmoides*). Tyler (1960) found a range of 3.5–4.0 g for *Trematomus borchgevinki* and a mean of 3.5 g for *Notothenia larsoni*, two Antarctic fishes.

Hematocrit

Hematocrit determinations were made in duplicate on 114 mountain whitefish (Table II). The range was 28.5–62.2% (mean 43.5%). Results were grouped on the basis of the time when fish were taken and ranges and means

TABLE II. Hemoglobin and hematocrit determinations; erythrocyte, leucocyte, and thrombocyte counts on mountain whitefish taken at various times (number of fish in parentheses; range = R, mean = M).

	Hemoglobin[a] (*g*)		Hematocrit (%)		Erythrocyte count (*millions*)		Leucocyte count (*thousands*)		Thrombocyte count (*thousands*)	
	R	M	R	M	R	M	R	M	R	M
April 1961	10.8–13.7	12.8 (6)	48.0–54.7	52.0 (5)	1.81–2.20	2.05 (4)				
July–Sept. 1961	7.0–12.9	10.4 (32)	31.7–57.2	41.1 (30)	1.12–1.87	1.35 (29)	0.83–25.5	7.1 (32)	3.3–35.2	17.8 (32)
July–Sept. 1962	9.6–13.0	11.1 (18)	33.2–62.2	50.0 (15)	1.03–1.46	1.28 (15)	1.5–15.9	6.5 (14)	5.4–35.2	17.0 (15)
Oct.–Dec. 1961	7.8–12.8	10.3 (38)	28.5–54.0	41.9 (40)	1.01–1.99	1.87 (38)	0.66–20.6	5.3 (37)	7.9–47.2	19.9 (38)
Jan.–Mar. 1962	7.8–12.7	10.5 (25)	30.7–52.8	43.2 (24)	1.04–2.34	1.46 (23)	0.36–5.6	2.1 (20)	0.87–28.0	13.2 (21)
Total:	7.0–13.7	10.6 (119)	28.5–62.2	43.5 (114)	1.01–2.34	1.57 (109)	0.36–25.5	5.4 (103)	0.87–47.2	17.6 (106)

[a]Cyanmethemoglobin technique.

TABLE III. Hemoglobin and hematocrit determinations; erythrocyte, leucocyte, and thrombocyte counts on mountain whitefish of various weights (number of fish in parentheses; total length (*cm*) in parentheses; range = R, mean = M).

Weight (g)	Hemoglobin[a] (g)		Hematocrit (%)		Erythrocyte count (*millions*)		Leucocyte count (*thousands*)		Thrombocyte count (*thousands*)	
	R	M	R	M	R	M	R	M	R	M
40–109 [18.5–25.5]	7.6–11.2	9.6 (11)	30.7–42.7	38.4 (12)	1.04–1.78	1.34 (11)	1.3–5.6	3.1 (10)	1.9–21.4	11.8 (10)
110–169 [26.5–29.0]	7.8–12.7	[illegible]	31.7–57.2	41.0 (29)	1.01–2.34	1.44 (30)	0.36–25.5	4.4 (29)	6.0–28.0	17.1 (30)
170–229 [26.0–31.5]	7.9–12.9	10.4 (18)	32.7–57.7	42.9 (17)	1.10–1.87	1.36 (16)	0.85–12.4	4.8 (17)	0.87–27.8	16.7 (17)
230–289 [29.0–33.0]	8.5–13.0	10.8 (15)	31.7–55.2	45.9 (14)	1.07–1.56	1.33 (15)	0.80–11.9	5.7 (14)	5.4–36.0	17.2 (15)
290–349 [30.5–37.5]	8.4–12.6	10.8 (12)	31.5–58.7	45.5 (13)	1.10–1.66	1.33 (11)	4.1–15.9	9.6 (10)	7.7–36.2	18.7 (11)
350–409 [30.5–32.5]	10.1–10.3	10.2 (2)	48.0	48.0 (1)	1.38–1.44	1.41 (2)	8.3–8.6	8.5 (2)	10.5–30.2	20.4 (2)
410–469 [37.0–40.0]	8.9–11.5	10.4 (5)	34.5–62.2	44.4 (3)	1.27–1.46	1.40 (4)	0.66–20.6	10.4 (4)	13.5–33.0	22.1 (4)
470–589 (none taken)										
590–650 [38.0–40.5]	7.0–9.7	8.4 (2)	39.5	39.5 (1)	1.12–1.20	1.16 (2)	1.2–2.9	2.1 (2)	21.3–47.2	34.3 (2)
Total:	7.0–13.0	8.4 (95)	20.7–62.2	43.1 (89)	1.01–2.34	1.37 (91)	0.36–25.5	5.4 (88)	0.87–47.2	17.3 (91)

[a]Cyanmethemoglobin technique.

TABLE IV. Hemoglobin and hematocrit determinations; erythrocyte, leucocyte, and thrombocyte counts on mountain whitefish arranged by age and sex (range = R, mean = M; number of fish in parentheses).

Age (*years*)	Hemoglobin (*g*)		Hematocrit (*%*)		Erythrocyte count (*millions*)		Leucocyte count (*thousands*)		Thrombocyte count (*thousands*)	
	R	M	R	M	R	M	R	M	R	M
1	8.5–11.2	9.8 (5)	32.7–42.5	37.5 (5)	1.22–1.61	1.42 (5)	1.3–5.6	3.3 (5)	1.9–19.3	11.4 (5)
2	7.6–12.6	10.9 (13)	33.5–57.7	44.9 (13)	1.03–1.63	1.28 (13)	1.5–25.5	4.8 (13)	2.7–24.9	13.9 (14)
3	9.0–13.0	10.8 (30)	32.0–57.0	45.3 (28)	1.07–2.34	1.39 (35)	0.36–15.2	5.1 (28)	0.87–27.5	15.4 (29)
4	8.9–12.5	10.9 (10)	34.5–62.2	48.6 (8)	1.18–1.57	1.31 (9)	0.85–15.9	8.2 (9)	8.0–36.2	19.5 (9)
5	9.5–9.7	9.6 (2)	36.7	36.7 (1)	1.20–1.27	1.24 (2)	2.9–20.6	11.8 (2)	13.5–47.2	30.4 (2)
6	7.0	7.0 (1)	39.5	39.5 (1)	1.12	1.12 (1)	1.2	1.2 (1)	21.3	21.3 (1)
Total:	7.0–13.0	10.6 (61)	32.0–62.2	43.5 (56)	1.03–1.63	1.35 (65)	0.36–25.5	5.5 (58)	0.87–47.2	15.9 (60)
Sex										
Male	7.9–13.0	10.9 (36)	31.7–62.2	47.4 (34)	1.07–1.87	1.40 (35)	0.36–24.9	5.8 (36)	0.87–36.0	15.2 (36)
Female	7.0–12.6	10.1 (51)	28.5–58.7	40.9 (50)	1.01–2.34	1.36 (54)	0.66–20.6	5.2 (49)	1.90–47.2	17.8 (55)
Total:	7.0–13.0	10.4 (87)	28.5–62.2	43.5 (84)	1.01–2.34	1.38 (89)	0.36–24.9	5.5 (85)	0.87–47.2	16.8 (91)

were computed. Five fish taken in April 1961 had a mean of 52.0% and 15 fish taken from July to September 1962 had a mean of 50.0%. All other groups had means slightly lower or higher than the total mean. When separated on the basis of weight, 12 fish (40–109 g, 1–2 years) had a mean of 38.4% and 77 fish (110–650 g, 2–6 years) had a mean of 43.6% (Table III). When results were grouped by sex (Table IV), males had a higher mean than females. The smaller fish had lower hematocrits. Young (1949) found no correlation between weights and hematocrit values of the opaleye.

A number of investigators have used the hematocrit test for various species of fishes. Normandeau (1962) obtained a mean of 43.9% in lake trout (*Salvelinus namaycush*) and a mean of 47.5% in brook trout (*Salvelinus fontinalis*). These are close to that found in mountain whitefish (43.5%). Haws and Goodnight (1962) gave a range of 17.0–41.5% (mean 29.4%) for the brown bullhead (*Ictalurus nebulosus*) and for the channel catfish (*Ictalurus punctatus*) a range of 15.0–47.0% (mean 27.9%). Murachi (1959) reported a range of 15.8–74.0% in young carp.

Erythrocytes

Count

Erythrocyte counts on 109 fish taken from April 1961 to September 1962 showed a range of 1.01–2.34 million erythrocytes/mm^3 of blood and a mean of 1.57 million (Table II). Results were grouped on the basis of time periods and ranges and means were determined. Thirty-eight fish taken from October to December 1961 had a higher mean than for other times of the year. This is associated with the spawning period of this fish (Brown, 1952). Katz (1951) found no variance in erythrocyte counts of prespawning silver salmon (*Oncorhynchus kisutch*). Males and females had similar means (Table IV). Means determined on 60 fish grouped according to age and on 91 fish for weight (Table III) showed only slight differences between the means of each group and the total mean.

Extreme differences in erythrocyte counts have been found for various species of fishes. Tyler (1960) reported a range of 0.66–0.80 million in *Trematomus borchgevinki* and a range of 0.380–0.389 million in *Notothenia larsoni*. Haws and Goodnight (1962) reported means of 2.16 million in the channel catfish and 1.27 million in the brown bullhead. Smith et al. (1952) compared erythrocyte counts in 10 species and found the lowest mean (1.24 million) in the goldfish (*Carassius auratus*) and the highest (1.88 million) in the warmouth (*Chaenobryttus gulosus*).

Size

Erythrocytes were measured on 30 mountain whitefish taken from August 1961 to September 1962 (Table V). The majority of mature cells was oval in shape but some were round (10 μ × 10 μ) and others long and narrow (range, 12 μ × 7 μ to 18 μ × 10 μ). Results were grouped according to weight, age,

TABLE V. Erythrocyte sizes (*microns*) of mountain whitefish arranged according to weights (*g*) and lengths (*cm*), ages (*year*), and sex.

Weight	Length	No. fish	Range	Mean
40–109	19–25	7	11.6 × 7.8–14.1 × 9.4	13.4 × 8.5
110–169	25–29	8	13.0 × 8.9–14.2 × 9.5	13.6 × 9.3
170–229	26–32	6	13.4 × 8.8–14.0 × 10.0	13.8 × 9.5
230–289	30–33	3	12.7 × 9.4–13.9 × 10.0	13.5 × 9.8
290–349	31–38	4	13.4 × 9.4–14.4 × 10.5	13.9 × 9.8
350–409		0		
410–469	37	1	13.6 × 9.4	13.6 × 9.4
470–609		0		
610–650	38	1	13.2 × 9.5	13.2 × 9.5
Total:		30	11.6 × 7.8–14.4 × 10.5	13.6 × 9.3

Age	No. fish	Range	Mean
1	3	11.6 × 7.8–13.4 × 8.3	12.8 × 8.0
2	7	13.2 × 8.2–14.0 × 10.0	13.6 × 9.2
3	13	13.0 × 8.8–14.2 × 10.0	13.8 × 9.4
4	6	12.7 × 9.4–14.4 × 10.5	13.7 × 9.7
5	0		
6	1	13.2 × 9.5–14.4 × 10.0	13.2 × 9.5
Total:	30	11.6 × 7.8–14.4 × 8.3	13.6 × 9.3

Sex			
Male	15	13.3 × 7.8–14.4 × 10.5	13.8 × 9.3
Female	15	11.6 × 7.8–14.4 × 10.0	13.5 × 9.3
Total:	30	13.3 × 7.8–14.4 × 10.5	13.6 × 9.3

and sex of fish. The 40–109-g weight group had the lowest mean. The 1-year-old fish had the lowest mean of all age groups. Erythrocyte means of males and females were similar.

The mean values of mature erythrocytes vary greatly between various species of fishes. The mean for grayling (13.2 μ × 9.5 μ) as reported by Lieb et al. (1953) is similar to that of mountain whitefish (13.2 μ × 9.5 μ) given in my study. Tyler (1960) found round cells (mean 11.0 μ) in *Trematomus borchgevinki*. Smith et al. (1952) reported almost round cells (mean 12.4 μ× 10.5 μ) in yellow bullhead (*Ictalurus natalis*) and oblong pointed cells (mean 11.1 μ × 7.7 μ) for warmouth bass.

LEUCOCYTES

COUNT

Leucocyte counts were determined on 103 mountain whitefish collected from July 1961 to March 1962 (Tables II–IV). The range was 360–25,500/mm^3

of blood with a mean of 5400. Results were grouped according to time periods taken and ranges and means were determined. The lowest mean was found in the January–March group. There was no great difference between the leucocyte counts of males and females. Yokayama (1947) reported a mean of 43,400 for yellow perch, but found no significant difference between the counts of males and females. No comparison was made between the leucocyte counts of mountain whitefish of various ages. Field et al. (1943) found means of 2105 in brook trout and 3675 in carp.

DIFFERENTIAL COUNT

Leucocyte differential counts were made on 109 mountain whitefish taken from July 1961 to September 1962 and ranges and means were determined for each cell type (Table VI). Very wide ranges were found for all cell types. Means for all fish showed the following percentages: small lymphocyte, 31.5; large lymphocyte, 2.4; lobed neutrophilic granulocyte, 58.5; band neutrophilic granulocyte, 6.8; metamyelocyte, 0.7; myelocyte, 0.1; basophil (mast) cells were seen in a few fish. No eosinophils or monocytes were found. The greatest percentage of lobed granulocytes was found in fish taken July–September and the lowest in January–March.

The percentages of lymphocytes and granulocytes (lobed and band granulocytes, metamyelocytes, myelocytes) were used for further comparisons which were made according to weight, age, and sex of fish. Specimens in the 40–109-g weight group had the lowest granulocyte value (Table VII). One-year-old fish generally had a lower percentage of granulocytes than older fish (Table VIII). Males had a lower granulocyte percentage than females (Table VIII). There was a higher granulocyte percentage in counts over 8100/mm^3 of blood.

Considerable differences were found between differential counts for mountain whitefish and those reported for other fishes. Counts for lake trout (Lieb et al., 1953) showed 91.2% lymphocytes, 8.2% monocytes, and 0.6% granulocytes. Yellow perch (Yokayama, 1947) had 67.7% lymphocytes and 32.4% neutrophilic granulocytes which he named "heterophiles." The predominant granulocyte was the myeloçyte. Eosinophils were occasionally found but no monocytes or basophils.

THROMBOCYTE

Counts were made on 106 mountain whitefish taken from April 1961 to September 1962 (Table II). The range was 870–47,200/mm^3 of blood and the mean 17,600. Results were grouped according to time periods (Table II), weight groups (Table III), age and sex (Table IV) of fish. Although there was a wide range, means were quite similar for most groups involving more than four fish. There were slightly lower thrombocyte counts on fish taken January–March, on fish in the 40–109-g weight group, and on 1-year-old specimens.

TABLE VI. Leucocyte differential counts on 109 mountain whitefish. Ranges and means in per cent for: small lymphocyte (SL); large lymphocyte (LL); lobed granulocyte (LG); metamyelocyte (ME); myelocytes (MY).

Time	No. fish	Range						Mean					
		SL	LL	LG	Band Gr.	ME	MY	SL	LL	LG	Band Gr.	ME	MY
July–Sept. 1961	32	1–82	0–10	14–96	0–48	0–25	0–10	21.6	1.6	66.3	8.9	1.3	0.3
July–Sept. 1962	22	0–62	0–3	24–94	0–16	0–15	0	23.8	0.2	69.4	5.7	0.9	0.0
Oct.–Dec. 1961	37	2–92	0–18	4–87	0–27	0–5	0.2	35.5	3.4	52.9	7.6	0.5	0.1
Jan.–March 1962	18	2–98	0–28	2–98	0–16	0	0	50.0	6.9	39.9	3.2	0.0	0.0
Total:	109	0–98	0–28	2–98	0–48	0–25	0–10	31.5	2.4	58.5	6.8	0.7	0.1

TABLE VII. Leucocyte differential count on mountain whitefish grouped by weight.

Weight (g)	Total length (*cm*)	No. fish	Range		Mean	
			Lymphocyte (%)	Granulocyte (%)	Lymphocyte (%)	Granulocyte (%)
40–109	18.5–25.5	12	20–96	4–80	65.3	34.7
110–169	25.0–30.0	30	2–90	2–98	41.8	59.2
170–229	26.0–31.5	16	1–79	21–99	29.7	70.3
230–289	29.0–34.0	16	0–90	10–100	23.4	76.6
290–349	30.5–37.5	14	0–94	6–100	22.8	77.2
350–409	31.0–33.0	2	10–19	81–90	14.5	85.5
410–469	37.0–40.0	3	2–62	36–98	24.0	76.0
470–589		0				
590–650	38.0–40.5	2	14–82	18–86	48.0	52.0
Total:		95	0–96	2–100	35.8	64.2

ERYTHROCYTE SEDIMENTATION RATE

Sedimentation rates were determined on 100 mountain whitefish taken from July 1961 to March 1962. The range was 0.0–3.0 mm and the mean 1.2 mm. Comparisons were made between results grouped according to weights and sex of fish. The 40–109-g weight group had the highest mean (1.4 mm). Mean values for males (0.9 mm) were similar to those of females (1.1 mm).

BLOOD CLOTTING TIME

Fifteen mountain whitefish were used for clotting time determinations. The range was 40–109 sec and the mean 72.1 sec. Smith et al. (1952) reported the lowest mean clotting time (10.8 sec) in goldfish and the highest (41.3 sec) in smallmouth buffalo (*Ictiobus bubalus*) for 10 species of fish studied.

MATURE AND IMMATURE BLOOD CELLS

ERYTHROCYTES

Mature erythrocytes were oval-shaped with oval nuclei which were not quite concentric with the outer edge of the cell showing a wider margin at the poles. The cytoplasm was orange-pink and the nucleus a deep purplish-blue. Small chromatin clumps were uniformly distributed in the nucleus. No nucleoli were seen. Degenerating erythrocytes had a dense purple, almost homogeneous (pycnotic) nucleus.

TABLE VIII. Leucocyte differential count on mountain whitefish arranged by age and sex; also grouped as to ranges of total leucocyte count.

		Range		Mean	
Age (*years*)	No. fish	Lymphocyte (%)	Granulocyte (%)	Lymphocyte (%)	Granulocyte (%)
1	7	58–96	4–42	75.9	24.1
2	19	4–80	20–97	38.3	61.7
3	36	0–98	2–100	26.5	73.5
4	11	1–62	38–99	23.8	76.2
5	1	14	86	14	86
6	1	82	18	82	18
Total:	75	0–98	2–100	34.3	65.7
Sex					
Male	38	0–98	2–100	41.4	58.6
Female	52	0–84	16–100	27.3	73.7
Totals:	90	0–98	2–100	33.3	66.7
Leucocyte count					
360–2,000	22	0–96	2–100	47.0	53.0
2,100–4,000	35	2–90	4–98	39.3	61.7
4,100–6,000	16	2–94	6–98	51.4	48.6
6,100–8,000	7	5–82	18–95	43.0	57.0
8,100–10,000	12	2–62	4–98	12.8	87.2
Over 10,000	18	0–96	4–100	17.7	82.3
Total:	110	0–96	2–100	36.4	64.6

Polychromatic erythrocytes were found occasionally in blood smears. These were shorter and wider than the mature cells. The cytoplasm stained light smoky-blue and the slightly elongated nucleus a dark purplish-blue with irregular chromatin clumps. These cells were found in all spleen and kidney imprints, but rarely in liver imprints.

Proerythrocytes were slightly larger than polychromatic erythrocytes and were round in shape as were their nuclei. The cytoplasm stained a dark basophilic color. The purplish-blue nucleus had a fine chromatin structure with only a few small chromatin clumps. Nucleoli were often visible. These cells were found in spleen imprints, but rarely in kidney and liver imprints. None was seen in blood smears. Many gradations were seen between the more

mature proerythrocytes and the "blast" stage. Erythroblasts were larger than erythrocytes and most proerythrocytes. The reticulate nucleus was surrounded by a narrow rim of very dark basophilic cytoplasm. Nucleoli were present and usually more easily recognized in the "blasts" than in other developmental stages. Erythroblasts were not found in blood, kidney, and liver smears, and only rarely in spleen imprints.

THROMBOCYTES

Mature thrombocytes were ovoid and slightly smaller than mature erythrocytes. Cytoplasm was very pale blue and usually showed only at the edge of the cell. The nucleus was less ovoid in the thrombocytes and had densly packed chromatin clumps. The greatest number of thrombocytes was found in kidney imprints. Degenerating thrombocytes were most numerous in blood smears and contained less cytoplasm having round, pycnotic nuclei.

Prothrombocytes were round with round nuclei. The cytoplasm stained medium blue and the dark blue nucleus had few chromatin clumps. Nucleoli were common. More prothrombocytes were found in kidney than in spleen imprints. None was seen in blood smears and liver imprints. No thromboblasts were found.

LYMPHOCYTES

Both small and large lymphocytes were seen in mountain whitefish blood with a greater percentage of small lymphocytes. The small cells were round and had round nuclei. A narrow rim of medium-blue cytoplasm surrounded the purplish-blue nucleus which had dense finely-clumped chromatin. Small pinkish granules were found in the cytoplasm of a few cells. Large lymphocytes had a greater amount of pale-blue cytoplasm and larger nuclei. These cells were more numerous in kidney imprints.

Prolymphocytes were similar in appearance to large lymphocytes except for finer chromatin structure of the nucleus and the presence of nucleoli. They were found most often in kidney imprints, occasionally in spleen imprints, and were absent from blood smears. Lymphoblasts were not seen.

Investigators have reported similarities in the various developmental forms of lymphocytes and thrombocytes (Lieb et al., 1953; Yokayama, 1947). Jordon and Speidel (1924) studied the blood of five teleosts and theorized that lymphocytes were precursors of thrombocytes. This was not substantiated in my study of mountain whitefish blood. Both cells may have developed from a common "blast" form, but were differentiated early in their development. Prolymphocytes had a lighter stained cytoplasm than prothrombocytes. Although some fish showed both types of immature cells in kidney and spleen imprints, others showed prothrombocytes in spleen imprints and prolymphocytes in kidney imprints. Degenerative thrombocytes tended to clump more readily than degenerative lymphocytes and more often showed blebs of cytoplasm and denser pycnotic nuclei.

NEUTROPHILIC GRANULOCYTES

Lobed neutrophilic granulocytes (mature forms) were the largest cells seen in mountain whitefish blood. These were round cells with a large amount of light pinkish-blue cytoplasm. The nucleus stained a dark, muddy-blue and had four or more lobes. Chromatin clumps were large and easily recognized. A number of variations existed between these cells. Some showed very fine pink granules in the cytoplasm and others had basophilic cytoplasm. Some fish had very large cells with nuclei containing as many as ten lobes. Because of these and other variations, the granulocytes of fishes have been given different names. Jordon and Speidel (1924) referred to these cells as granulocytes, pseudoeosinophils, and special leucocytes. Yokayama (1947) found a 2–4-lobed nucleus in the granulocytes of yellow perch and termed these cells "heterophiles." The granulocytes of mountain whitefish more closely resembled the typical neutrophilic granulocytes of human blood. These were numerous in blood smears and kidney imprints. Few were seen in liver and spleen imprints.

Band neutrophilic granulocytes resembled the lobed cells except for the band shape of the nucleus. These cells were also numerous in kidney imprints and some were found in most blood smears.

Metamyelocytes were of similar size to the mature forms. They were easily recognized by the slightly indented shape of the nucleus. No nucleoli were seen. These cells were most often found in kidney imprints, sometimes in blood smears, and rarely in liver and spleen imprints.

Myelocytes were the same size or larger than metamyelocytes and had a more basophilic cytoplasm. The nucleus often stained lighter blue and was of finer chromatin structure than in myelocytes and had visible nucleoli. Myelocytes were numerous in kidney imprints and were occasionally found in blood smears.

Granuloblasts were larger than the myelocytes. This cell type had a narrow rim of dark basophilic cytoplasm with few granules and the large reticulate nucleus stained pale purplish-blue. Nucleoli were larger than those seen in myelocytes. Only kidney imprints showed granuloblasts.

BASOPHIL (MAST) CELLS

These were large, round cells which contained many large, spherical, bluish-black granules that almost completely covered the nuclei. They were numerous in kidney imprints and rare in blood smears. Basophils were not reported for yellow perch (Yokayama, 1947) or for grayling and lake trout (Lieb et al., 1953). Bolton (1933) reported basophils in the entire alimentary canal of six salmonids, but only in the intestinal muscularis and serosa of mountain whitefish. No mention was made of blood or kidney examinations.

UNIDENTIFIED CELLS

These large mononuclear cells had round nuclei and pale blue cytoplasm which contained large, dark-blue, rod-like or spherical granules. In some fish these cells were numerous in kidney imprints and common in blood smears.

They were almost identical to those described for human blood by Wintrobe (1962) who found them in myelogenous and monocytic leukemias. Photographs of these leukemic cells are shown in Sandoz Atlas of Haematology (1952). Similar cells in mountain whitefish blood were possibly toxic myelocytes.

Laboratory-Induced Anemia

Twenty mountain whitefish were maintained in the laboratory for 7–127 days following the first blood sampling. Blood was taken a second time from these fish and tests were made using methods previously described. Kidney, liver, and spleen imprints were made. All fish at the second sampling appeared anemic, had hemorrhagic spots under the skin, and possible fungus infections on fins and body surfaces. The mean values for these anemic fish are shown in Table IX. Hemoglobin and hematocrit means and erythrocyte counts were considerably lower in anemic fish than for the 119 mountain whitefish

Table IX. Hematological values on laboratory-induced anemic mountain white fish compared with means for all other whitefish.

	Anemic fish			Other whitefish
Values	No. fish	Range	Mean	Mean
Hemoglobin (g)	20	3.8–9.2	6.8	10.6
Hematocrit (%)	20	13.0–37.2	28.6	43.5
Erythrocyte count (*millions*)	20	0.79–1.35	1.05	1.57
Leucocyte count (*thousands*)	20	2.7–16.7	6.6	5.4
Thrombocyte count (*thousands*)	20	5.2–53.2	22.0	17.6
Erythrocyte sed. rate (*mm*)	18	1.0–3.0	1.7	1.2
Leucocyte differential count	20			
Lymphocyte (%)		2–94	26.4	33.9
Granulocyte (%)		6–98	73.6	66.1

described. Means for leucocyte and thrombocyte counts, erythrocyte sedimentation rates, and granulocyte percentages were higher in the anemic fish. Infection may have caused the higher sedimentation rate, leucocyte counts, and granulocyte percentages. Schumacher et al. (1956) reported higher values for sedimentation rates in brook trout with furunculosis. Toxic chemicals can also cause granulocytosis as reported by Yokayama (1947) who injected yellow perch with turpentine. Blood smears from anemic fish had many hypochromic, microcytic, and immature erythrocytes. Polychromatic erythrocytes, proerythrocytes, and erythroblasts were most numerous in spleen imprints. Mitotic erythrocytes were seen only in spleen imprints. Mature and immature thrombocytes were numerous in kidney and sometimes in the spleen imprints. Many immature and mitotic granulocytes were found in kidney and sometimes in spleen imprints. Basophil (mast) cells were numerous in most kidney and occasionally in spleen imprints. Unidentified mononuclear cells were numerous on some blood and kidney slides.

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The Effect of Physical Conditioning on the Metabolism of Lactate, Phosphate, and Glucose in Rainbow Trout, *Salmo gairdneri*[1]

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ABSTRACT

Physiological effects of physical conditioning to water current were studied on three groups of 1½-year-old rainbow trout, *Salmo gairdneri*, acclimated to 4 C. Group one (control) was raised in still water. Groups two and three were conditioned to water velocities of 20 cm/sec and 40 cm/sec, respectively, for 16 days before sampling. Muscle and plasma samples were collected before exercise and four times during subjection to 15 min of forced swimming at 53.4 cm/sec and eight times during a 24-hr recovery period. Conditioning significantly delayed the point of fatigue during forced exercise: the unconditioned fish were fatigued after about 5 min swimming, group two after about 10 min swimming, and group three at about 15 min.

Physically conditioned trout showed significantly higher muscle and plasma lactate levels when fatigued, and more rapid removal of lactate from muscle and plasma during recovery from fatigue, than unconditioned trout. Exercise resulted in parallel oscillating concentration fluctuations of tissue phosphate and significant increases in concentrations of plasma phosphate in both conditioned and unconditioned fish. Plasma glucose showed no significant change during exercise but rose slightly during the recovery of all groups.

INTRODUCTION

Muscular activity in fish produces a pattern of changes in carbohydrate metabolism similar to that of higher vertebrates. Extensive studies by Black and his colleagues with several salmonid species have shown that muscle glycogen is rapidly mobilized during exercise and that substantial amounts of lactate and pyruvate appear immediately in the muscle (Black et al., 1959, 1960, 1961; Parker and Black, 1959). Following cessation of exercise, lactate diffuses relatively slowly from the muscle resulting in a prolonged elevation of the blood lactate concentration lasting more than 12 hr if exercise was severe (Black et al., 1961).

Studies with trout have indicated that maximal attainable swimming speed and physical stamina are increased by physical conditioning (Brett et al., 1958; Hochachka, 1961; Bainbridge, 1962). Hochachka found that conditioned fish used more of their muscle glycogen reserves than unconditioned fish. Conditioned fish sustained an oxygen debt three times that of unconditioned fish indicating a more extensive utilization of nonoxidative sources of energy. Since even conditioned fish were unable to use all of their glycogen

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reserves, Hochachka suggested that stamina was limited not by the amount of energy reserves present but by the excessive accumulation of lactic acid during exercise together with a limited capacity of muscle to buffer this acid. Presumably therefore, the increase in stamina provided by physical conditioning is associated at least in part with an increased tolerance for lactic acid accumulation, perhaps because buffering capacity is improved.

The present study was undertaken especially to study the pattern of lactic acid concentration changes during exercise of conditioned and unconditioned trout. Measurements of plasma glucose and plasma and muscle inorganic phosphate levels also were made as additional potential indicators for the evaluation of the physiological effects of physical conditioning.

METHODS

Maintenance of Experimental Fish

The experiments reported were carried out during the summer of 1963 at the Provincial Fish Hatchery in Calgary, Alberta. Rainbow trout, *Salmo gairdneri*, were hatched in February 1962, transported to outdoor rearing ponds in June 1962, and returned to the hatchery in the spring of 1963. Average size of the trout was 46 g. The fish were held in three identical circular tanks, each 3.94 m in diameter. Water entered each tank under pressure from five jets mounted horizontally over the water surface, and was removed by way of a standpipe in the center of the tank. All groups were fed Clark's "New Age" pellet diet at equal rates. The fish were not fed the day samples were taken. The water was saturated with oxygen and maintained at a temperature of 4 C.

Current acclimation was begun May 16, 1963. A constant rotating water current was created in two of the three circular tanks by adjusting the angle of the inflowing jets of water. Two groups of trout, hereafter referred to as group two and group three, were subjected to gradually increasing water current over an 8-day period. The final surface current velocity for group two was 20 cm/sec and for group three 40 cm/sec. With gradual conditioning over 8 days, all trout were able to maintain position in the current indefinitely without increased mortality. A control group, group one, was held in low current and showed no current orientation.

Exercise and Sampling Procedure

Sampling was begun 16 days after all fish had been established in their final current velocities. A rotating exercise tank (Fig. 1) was positioned adjacent to a circular conditioning tank. Trout were individually exercised by transferring them by net from the conditioning tanks to the channel of water rotating at 53 cm/sec in the channel center. All fish endeavored to maintain a constant position in the tank with respect to the operator. Group one trout (controls) fatigued after approximately 5 min and group two at approximately 10 min after which they were unable to maintain position in the current.

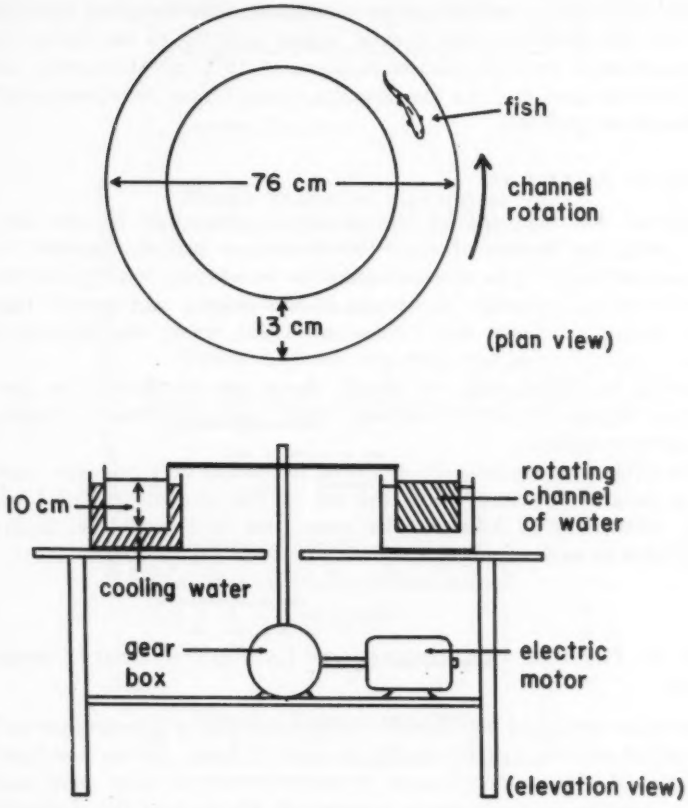

FIG. 1. Rotary fish tank for strenuously exercising fish at a constant speed.

Group three trout fatigued after approximately 15 min. Fish were sampled at 2, 5, 10, and 15 min during a 15-min exercise period. To achieve uniform exercise for all three groups, group one and group two trout had to be mechanically prodded into maintaining position during the more prolonged intervals of exercise. Eventually swimming activity in group one and group two fish became so weak that it was necessary to apply the stimulus with a small screen which partially supported the fatigued fish in the current.

Following exercise, fish were water-brailed to a separate covered trough (8.85 m long × 32 cm wide × 16.5 cm deep) and left undisturbed in darkness. Fish were sampled at 0.25, 0.5, 1, 2, 4, 8, 16, and 24 hr following exercise (recovery period). A 400-μliter blood sample was taken from the ventral aorta with a heparinized tuberculin syringe while holding the fish under water. The plasma was immediately separated in a microcentrifuge. The fish was killed

by spinal transection and a sample of white muscle weighing approximately 0.1 g was removed from the epaxial region anterior to the dorsal fin. This was immediately homogenized with 10 cc of 10% trichloroacetic acid in a Serval homogenizer, and the homogenate stored frozen for subsequent lactate and phosphate analyses.

Analytical Procedure

Glucose was analysed by the glucostat method of Keston and Teller (1956), using the Beckman-Spinco 150 ultramicro analytical system. Samples were analysed within 1 hr after collection to avoid error from glycolysis.

Analysis for inorganic phosphate in the plasma and muscle tissue was by the method of Fiske and Subbarow (1925), using the Beckman-Spinco system.

Lactate in the plasma and muscle tissue was determined by the micro-method of Scholander and Bradstreet (1962), using an Hitachi Perkin-Elmer 139 spectrophotometer.

The data were statistically analysed by means of a one-way analysis of variance program prepared with the aid of the Department of Computing Science, University of Alberta. The mean and 95% confidence interval for each variable at each sampling time were plotted (Fig. 2–12).

RESULTS

Effect of Physical Conditioning on Endurance During Strenuous Activity

The three groups of fish showed marked differences in resistance to fatigue during forced exercise in the rotating channel of water. Group one trout (controls) fatigued after approximately 5 min after which they were unable to maintain position in the current unassisted. Group two fish fatigued after approximately 10 min. Group three fish showed the most stamina and fatigued after approximately 15 min of exercise. These differences in endurance were marked and always observed during the exercise period but were not critically timed.

Muscle Lactate

The pre-exercise levels of muscle lactate in the three groups correspond directly with degree of physical conditioning (Fig. 2), although the differences are not highly significant (significant at the 90% probability level). Higher pre-exercise muscle lactate levels in the conditioned groups are an expected consequence of the constant swimming effort expended by these groups in the current of the conditioning tanks.

With the onset of strenuous activity, muscle lactate increases immediately (Fig. 3). The overall average increase in the first 2 min of exercise is 180.5%. Control group one muscle lactate rises from 63.5 mg/100 g wet wt to a peak

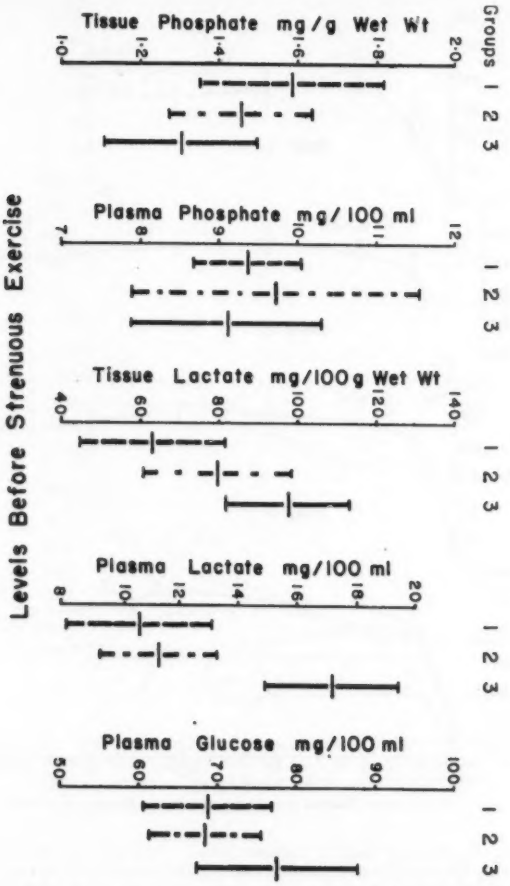
Levels Before Strenuous Exercise
Tissue Phosphate mg/g Wet Wt
1·0 1·2 1·4 1·6 1·8 2·0
Groups 1 2 3
Plasma Phosphate mg/100 ml
7 8 9 10 11 12
1 2 3
Tissue Lactate mg/100g Wet Wt
40 60 80 100 120 140
1 2 3
Plasma Lactate mg/100 ml
8 10 12 14 16 18 20
1 2 3
Plasma Glucose mg/100 ml
50 60 70 80 90 100
1 2 3

FIG. 2. Pre-exercise concentrations of the metabolites analysed: muscle phosphate, plasma phosphate, muscle lactate, plasma lactate, and plasma glucose. Sample size was 20 fish for each group. The 95% confidence interval is shown.

of 264.0 mg after 5 min then falls during the remaining 10 min of forced exercise. Group two muscle lactate rises from 78.8 mg to a maximum level of 290.3 mg after 10 min, followed by a slight decrease during the remaining 5 min of forced exercise. Group three shows a continual rise from 96.8 mg/100 g wet wt to a peak of 322.18 mg/100 g after 15 min exercise. During the exercise period, there is a separation in muscle lactate concentrations of the three groups, with significantly greater lactate concentrations appearing in the muscle of conditioned fish. These differences correspond directly to observed differences in stamina of the three groups.

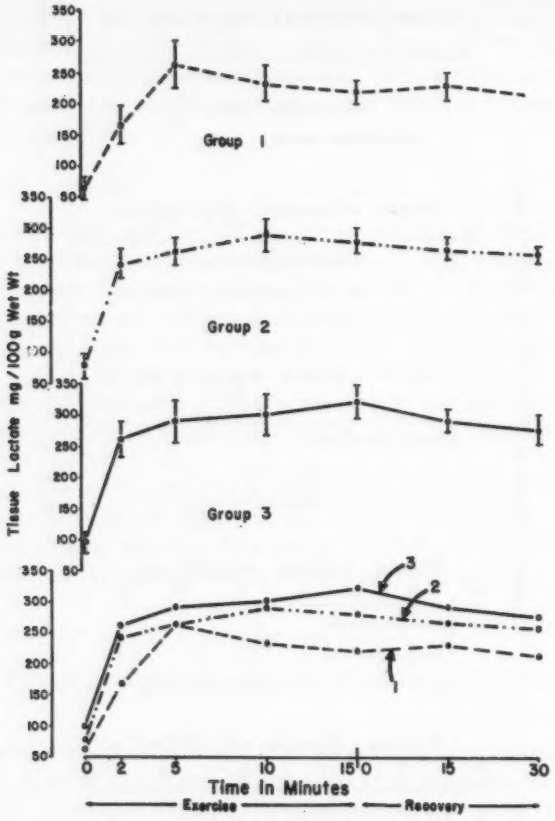

FIG. 3. Muscle lactate concentrations (mg/100 g wet wt) during 15 min of strenuous exercise. Sample size for each group: 20 fish for initial sampling time (pre-exercise), and 10 fish for each sampling time during exercise and recovery period. The 95% confidence interval is shown.

Muscle lactate began to decline immediately following cessation of activity (Fig. 4). In all three groups, removal was initially rapid, then diminished gradually in a pattern very similar to that described by Black et al. (1962) for rainbow trout. However, the elimination of lactate is substantially more rapid in the conditioned groups than in the unconditioned control group one. The percentage decreases during the first 8 hr of recovery are 71% for group three, 64% for group two, and 59% for group one.

Thus, both the total accumulation of muscle lactate during exercise and its subsequent rate of removal during recovery, vary directly with degree of physical conditioning.

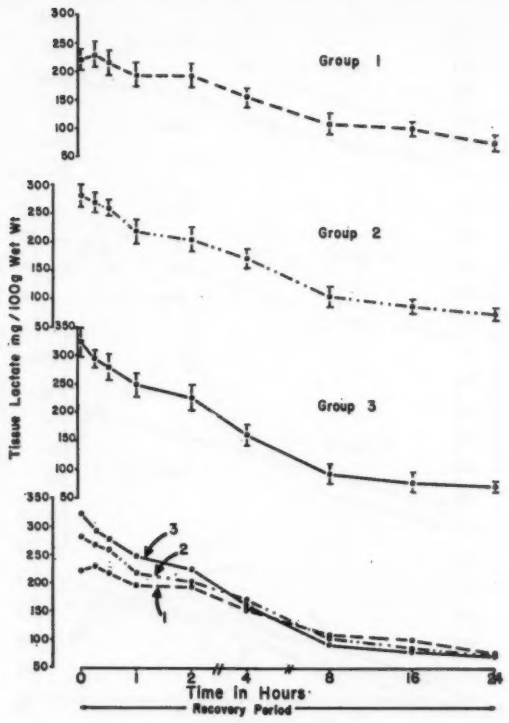

FIG. 4. Muscle lactate concentrations (mg/100 g wet wt) during 24 hr of recovery. Sample size for each group was 10 fish for each sampling time. The 95% confidence interval is shown.

PLASMA LACTATE

The plasma lactate level of the conditioned fish was increased significantly by their pre-exercise activity (Fig. 2). However, all groups have plasma lactate levels of approximately 17% of their muscle lactate concentrations.

During forced activity, the plasma lactate rises gradually and remarkably smoothly in all three groups of trout (Fig. 5). This increase, the source of which is probably entirely skeletal muscle lactate, averages 3.08 mg/100 ml/min during the 15 min exercise period. Following exercise, the plasma lactate continues to increase, though more slowly than during exercise and at a rate that varies with degree of physical conditioning. Group three plasma lactate increases at 0.54 mg/min during the first 30 min of recovery as compared to an increase rate of 0.27 mg/min for group one (Fig. 5).

Peak plasma lactate concentrations were measured at 2 hr of recovery for all groups (Fig. 6). At that time group three plasma lactate (126.7 mg/100 ml) is significantly greater than that of group one (89.9 mg/100 ml).

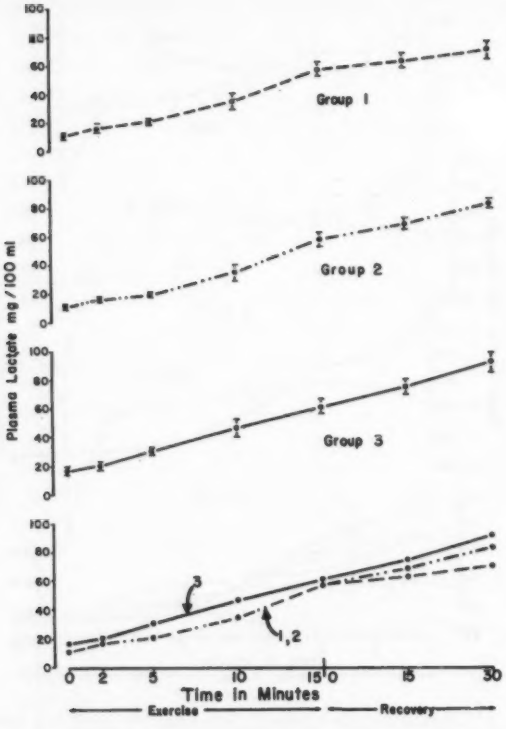

FIG. 5. Plasma lactate concentrations (mg/100 ml) during 15 min of strenuous exercise. Sample size for each group: 20 fish for initial sampling time (pre-exercise), and 10 fish for each sampling time during exercise and recovery period. The 95% confidence interval is shown.

Group three shows the most rapid rate of plasma lactate removal (Fig. 6). After 8 hr of recovery group three is significantly lower than group one (30.4 and 45.4 mg/100 ml, respectively). This trend continues until 24 hr recovery at which time there is no statistically significant difference between group means. At 24 hr plasma lactate has returned to pre-exercise levels for groups one and two while group three (9.6 mg/100 ml) has a level significantly lower than the pre-exercise level (17.0 mg/100 ml).

The effect of conditioning is manifested in the higher exercise-induced plasma lactate levels and in the more rapid disappearance of lactate from circulation during recovery of the conditioned fish. Throughout the exercise and recovery periods, plasma lactate concentrations are substantially below those of skeletal muscle, indicating a gradual transfer of this product to the general circulation.

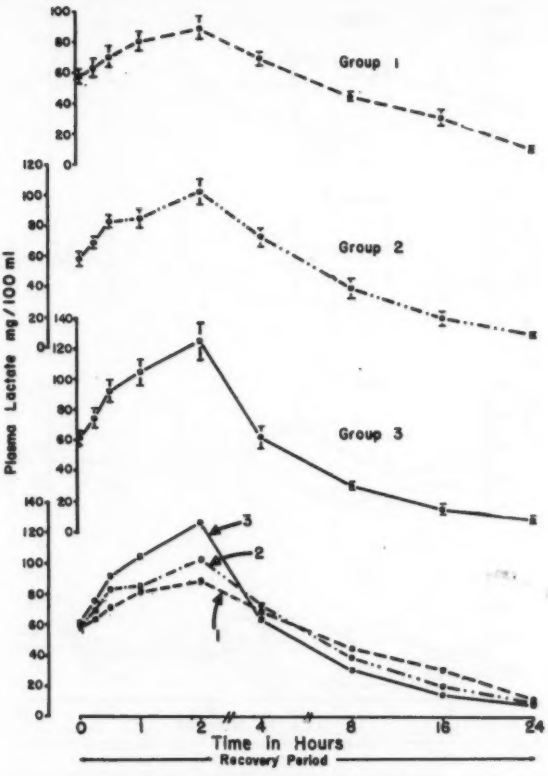

FIG. 6. Plasma lactate concentrations (mg/100 ml) during 24 hr of recovery. Sample size for each group was 10 fish for each sampling time. The 95% confidence interval is shown.

PLASMA GLUCOSE

Physical conditioning had no significant effect on the pre-exercise levels of plasma glucose (Fig. 2). Figures 7 and 8 show the effect of strenuous exercise on plasma glucose levels. Despite the high variability of plasma glucose amongst individual fish and the resulting instability of the group means during exercise, a trend toward increasing plasma glucose concentrations following exercise can be distinguished. Group one has levels at 4, 8, 16, and 24 hr recovery and group two has levels at 4, 8, and 24 hr recovery that are significantly higher than their pre-exercise levels (Fig. 7 and 8).

The effect of strenuous exercise on plasma glucose is to cause an overall rise in concentration during recovery. This post-exercise increase appears to be greater in the unconditioned fish (group one) but because of high variability within the groups, the differences are not statistically significant.

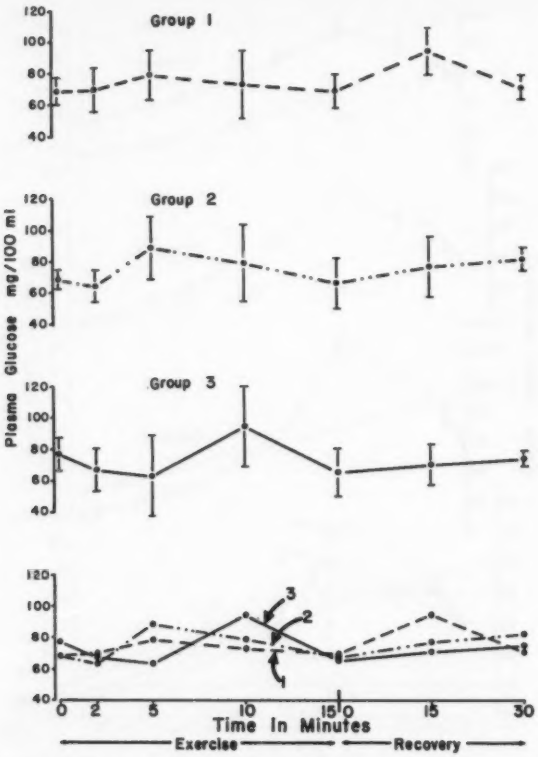

FIG. 7. Plasma glucose concentrations (mg/100 ml) during 15 min of strenuous exercise. Sample size for each group: 20 fish for initial sampling time (pre-exercise), and 10 fish for each sampling time during exercise and recovery period. The 95% confidence interval is shown.

MUSCLE INORGANIC PHOSPHATE

Physical conditioning produced no statistically significant difference in the pre-exercise levels of muscle phosphate (Fig. 2). During exercise, the concentration of muscle phosphate fluctuates markedly (Fig. 9). The fluctuations are oscillating in form and show a remarkable similarity of pattern in all three groups. At any given sampling time, the differences between group means are not statistically significant, but the changes between times within a group are frequently of statistical significance. In group one the phosphate concentrations at 5 min (1.79 mg/g wet wt) and 15 min (1.72 mg/g) are significantly greater than those at 2 min (1.32 mg/g) and 10 min (1.29 mg/g) (Fig. 9). Similarly, group two phosphate concentrations at 5 min (1.66 mg/g wet wt) and 15 min (1.56 mg/g) are significantly greater than those at 2 min (1.08 mg/g) and 10 min (1.05 mg/g). Muscle phosphate concentrations were more

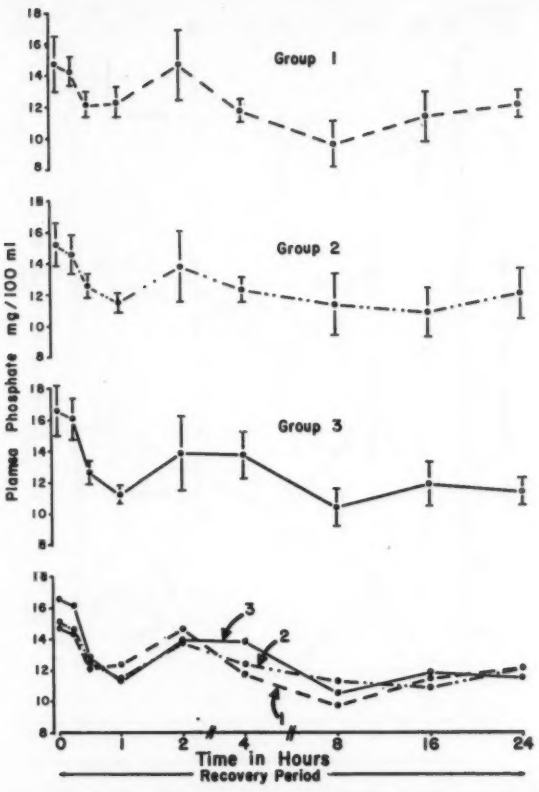

FIG. 8. Plasma glucose concentrations (mg/100 ml) during 24 hr of recovery. Sample size for each group was 10 fish for each sampling time. The 95% confidence interval is shown.

variable among individual fish of group three and the changes in mean concentrations are as a result not statistically significant from one another. The pattern of change in group three is nevertheless essentially the same as that of groups one and two. Following the cessation of forced exercise, muscle phosphate concentrations of all three trout groups showed a similar pattern of stabilization followed by a gradual decline to pre-exercise levels (Fig. 10). Phosphate concentrations in group two and three rose somewhat during the first 15 min of recovery, then fell gradually. The lowest concentrations were measured after 8 hr of rest (Fig. 10). After 24 hr of recovery the levels were not significantly different from the pre-exercise levels and the muscle phosphate concentrations of all three groups are nearly identical.

In summary, exercise produces large oscillations in tissue phosphate concentrations which stabilize during recovery at levels somewhat above the pre-

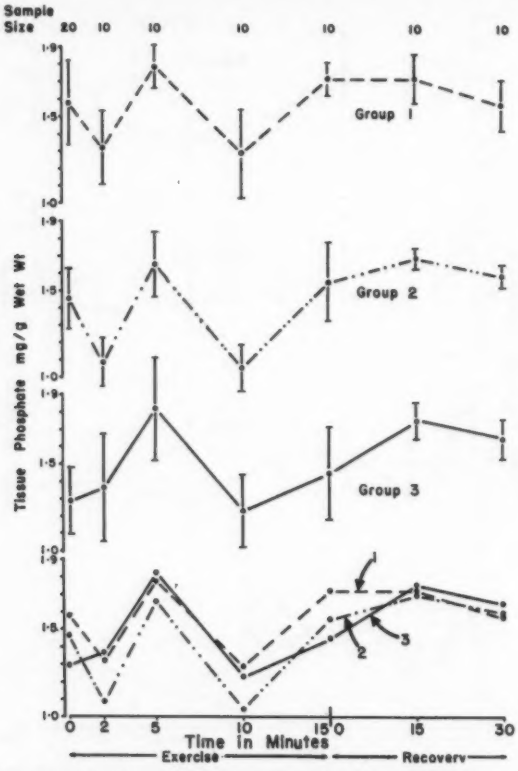

FIG. 9. Muscle phosphate concentrations (mg/g wet wt) during 15 min of strenuous exercise. Sample size for each group: 20 fish for initial sampling time (pre-exercise), and 10 fish for each sampling time during exercise and recovery period. The 95% confidence interval is shown.

exercise level. The decline to pre-exercise levels is gradual over a period of several hours. No significant difference in tissue phosphate between the three group means was observed.

PLASMA PHOSPHATE

The pre-exercise levels of plasma phosphate were not altered significantly by physical conditioning (Fig. 2). However, exercise was accompanied by a rapid and significant increase in plasma phosphate levels of all three groups of trout (Fig. 11 and 12). All groups showed the same general pattern of response: plasma phosphate increased sharply during the first 2 min (average rise 27.8%), then declined slightly at 5 min. Between 5 and 10 min, the concentrations again increased (average increase 40.0%) to reach peak or near-peak levels at 10 min. Further exercise (between 10 and 15 min) had no sig-

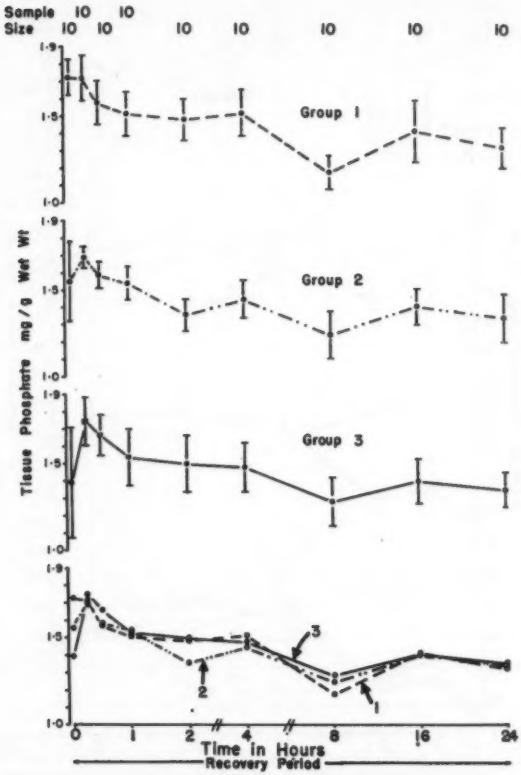

FIG. 10. Muscle phosphate concentrations (mg/g wet wt) during 24 hr of recovery. Sample size for each group was 10 fish for each sampling time. The 95% confidence interval is shown.

nificant effect on plasma phosphate concentrations. Following exercise, plasma phosphate at first fell rapidly. Thereafter the concentration fluctuated somewhat and never completely returned to the pre-exercise levels (Fig. 12).

In summary, exercise caused a significant increase in plasma phosphate. As with tissue phosphate, there is no difference between the three groups in the pattern of plasma phosphate change following exercise, and therefore no detectable effect of physical conditioning.

DISCUSSION

This research has demonstrated that physical conditioning greatly increased stamina of rainbow trout to forced exercise. This increase is associated with demonstrable differences between conditioned and unconditioned fish

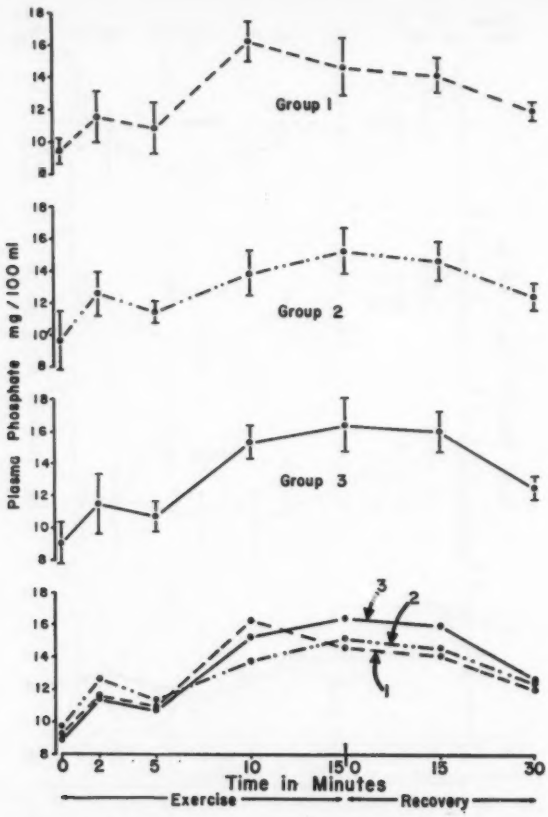

FIG. 11. Plasma phosphate concentrations (mg/100 ml) during 15 min of strenuous exercise. Sample size for each group: 20 fish for initial sampling time (pre-exercise), and 10 fish for each sampling time during exercise and recovery period. The confidence interval is shown.

with respect to quantity of lactate produced during exercise and its rate of removal from muscle and blood following exercise. Glycolysis is a major source of ATP consumed during strenuous exercise. This is especially so in fish where limitations of the respiratory system and a meager blood flow to the trunk musculature make impossible any substantial contribution of oxidative phosphorylation to replenishment of energy stores during exercise. Lactic acid rapidly appears in the muscle and plasma of fish; the rate of appearance and total concentrations reached depend on the intensity of exercise. This relation has been demonstrated by Black (1955, 1957a, b, c), Black at al. (1959), Black et al. (1960), Miller et al. (1959), Miller and Miller (1962), and Nakatani (1957) working with salmonids and Secondat and Diaz (1942) working with cyprinids.

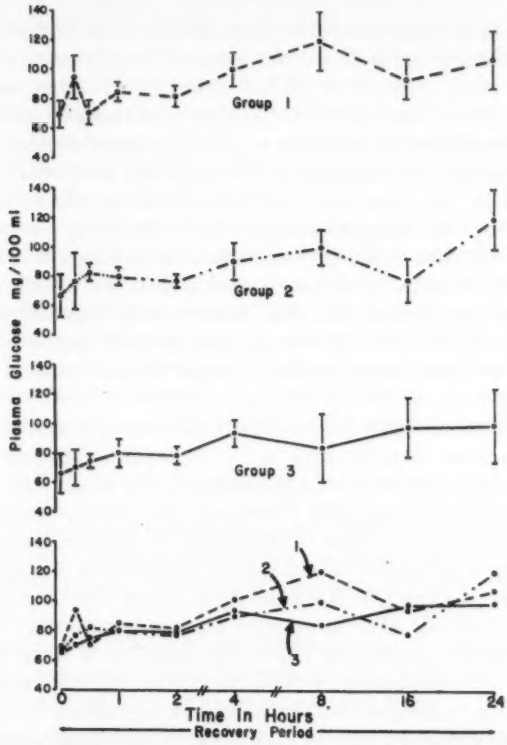

FIG. 12. Plasma phosphate concentrations (mg/100 ml) during 24 hr of recovery. Sample size for each group was 10 fish for each sampling time. The 95% confidence interval is shown.

In the present research it is assumed that the energy required during exercise is equivalent to the amount of work performed. It is evident that the conditioned group three accomplished the most work because significantly more lactate was produced (Fig. 5 and 6). This is in agreement with Hochachka's (1961) observation that physically conditioned trout utilized more of their glycogen reserves during strenuous exercise. As lactate is diffusible across the cell membrane, the concentration in the plasma will shift in rough proportion to changes in its concentration in muscle. As expected, the conditioned group three has the highest plasma lactate concentration after 15 min of strenuous exercise. An analogous situation was reported by Robinson and Harmon (1941) who found that man's ability to accumulate lactate during anaerobic metabolism increased with physical training. It is apparent that physically conditioned trout can continue activity in the presence of higher muscle lactate levels for a longer period than can unconditioned trout. However, it is not possible to provide a definite answer to the suggestion of Hochachka (1961) that lactate acts as a limiting factor in sustained activity of trout. Increased lactic acid

concentrations have been shown to correspond to an immediate decrease in muscle glycogen stores and a delayed decrease in liver glycogen stores with forced exercise (Black et al., 1960, 1961; Miller et al., 1959; Miller and Miller, 1962). Hochachka (1961) found that physically conditioned and unconditioned trout mobilize nearly equal amounts of liver glycogen during the early period of recovery following exercise. Hence, it is expected that nearly equal amounts of plasma glucose will enter the circulation. Group three showed the least significant increase in plasma glucose during recovery, suggesting a more rapid removal of glucose from the circulation and a larger utilization in a more active metabolic system. A possible explanation is found in the work of MacLeod (quoted by Black et al., 1961), who reported a deficiency of hexokinase in muscle of steelhead trout (*Salmo gairdneri*). This suggests that physically conditioned trout have larger concentrations and/or a more active form of muscle hexokinase.

Exercise produced large oscillating fluctuation in muscle inorganic phosphate concentrations. The changes are statistically significant and all three groups exhibit the same sequence of changes with time. The pattern is suggestive of a feedback system with overshoot. We have adapted the principles of Reiner (1959) for a possible explanation of this behaviour which is as follows. Before strenuous exercise the system is in a state of equilibrium and the rate constant (k_1) for the utilization of ATP equals the rate constant (k_2) for ATP synthesis (Fig. 13). At the start of strenuous activity the rate constant (k_1) will be increased resulting in the rapid depletion of stored ATP and creatine phosphate. The utilization of ATP for the energy-requiring processes will be accompanied by a corresponding increase in adenosine diphosphate (ADP) and inorganic phosphate. These in turn become available to react in the coupling mechanisms and permit respiration to proceed. As the anabolic processes are capable of an excess production of ATP the system over-compensates and concentrations of ADP and inorganic phosphate are greatly reduced. Synthesis is thus retarded. Not until the concentrations of ADP and inorganic phosphate increase are the anabolic processes again activated, with the result that concentrations of inorganic phosphate show significant fluctuations. Webb (1963) states that cyclic regenerative, and feedback enzyme systems are particularly prone to overshoot and fluctuation.

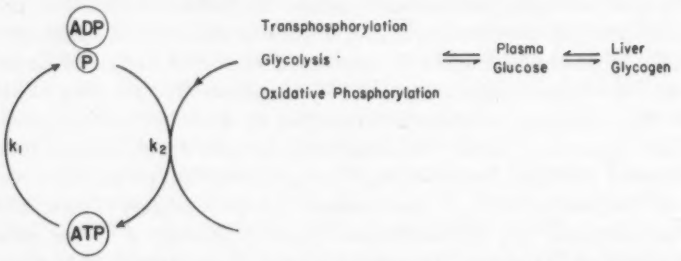

FIG. 13. Metabolic pathways for the generation and storage of energy: cyclic model of ATP metabolism and reversible pathways of plasma glucose.

Because of significant cycling in muscle phosphate concentration with time during exercise, this quantity is a poor indicator of the degree of fatigue. Plasma phosphate increases gradually during exercise to levels significantly above pre-exercise levels, thus providing a better indicator of exercise. The source of this increased plasma phosphate is almost certainly ATP, as suggested by the work of Jones and Murray (1957) who demonstrated an 80% decrease in ATP after exhaustion of *Gadus callarias*.

The primary effect of physical conditioning may be on enzyme systems involved in the mobilization of energy supplies for strenuous activity and in the rapid recovery from fatigue. For practical benefit to fisheries management the most efficient water temperature and the minimum time required for maximum physical conditioning under various water currents must be studied. It would be of interest to investigate the stamina response and the physiological effects of exercising physically conditioned fish at various temperatures. Such information would be of importance in fish stocking programs.

SUMMARY

One and one-half-year-old rainbow trout, *Salmo gairdneri*, were acclimated at 4 C and physically conditioned to water currents of 40 cm/sec (group three) and 20 cm/sec (group two). The controls (group one) were raised in low water current.

Exercising trout at 54.3 cm/sec disclosed that group one fatigued after approximately 5 min, group two after approximately 10 min, and group three at approximately 15 min of forced strenuous swimming.

Conditioning significantly increased the pre-exercise concentration of plasma lactate. There were no statistically significant differences in the pre-exercise levels of muscle lactate, phosphate, plasma phosphate, and plasma glucose.

Exercise increased the concentration of muscle lactate, with peak concentrations being established at the approximate times of fatigue for all groups.

Plasma lactate increased during exercise, and continued to increase after exercise, reaching a peak after 2 hr of rest.

Physical conditioning of rainbow trout enables them to produce higher concentrations of lactate during extended periods of exercise and remove lactate more rapidly during recovery.

Plasma glucose of groups one and two increased significantly during recovery following exercise.

The effect of exercise on muscle phosphate was to induce significant ocillating concentration fluctuations which subsided during recovery.

Plasma phosphate concentration increased significantly with exercise.

No statistically significant differences amongst groups in the concentrations of muscle and plasma phosphate were produced by physical conditioning and exercise.

ACKNOWLEDGMENTS

The authors are grateful to Mr M. J. Paetz, Mr A. C. Sinclair, and the staff of the Province of Alberta Calgary Fish Hatchery for the generous provision of laboratory space in the hatchery, and fish for this study. We wish to thank Dr E. C. Black and Mr Don Stevens for their critical reading of the manuscript. Financial support was provided by the Alberta Department of Lands and Forests, Fish and Wildlife Division, and by National Research Council of Canada Research Grant A-1415 to the junior author.

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Stomach Contents of Salmon and Steelhead Trout in the Northeastern Pacific Ocean[1]

By R. J. LeBrasseur

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ABSTRACT

Stomachs of pink, chum, sockeye, and coho salmon and steelhead trout caught during the summer of 1958 in gillnets fished overnight in the northeastern Pacific Ocean contained mainly zooplankton (*Limacina*, amphipods, copepods, and euphausiids), squid, and fish. Except for sockeye, there were no differences in contents related to fish size or state of maturity. Differences were found between species in the kinds of stomach contents present. The predominant organisms were amphipods and fish in pink salmon, crustaceans in immature sockeye, euphausiids and squid in maturing sockeye, euphausiids, fish, and squid in coho, and fish and squid in steelhead stomachs. The stomach contents of chum salmon were notable in that most of their contents were too well digested to identify. Comparison with the findings of workers in the northwestern Pacific showed no significant differences in the kinds of stomach contents, however, a greater amount of material was present in the stomachs they examined. The contents of stomachs from fish taken in various ocean domains were compared. Greater differences were noted in the stomach contents of fish from different domains than from different species. It is suggested that feeding is associated more with availability rather than with preferences for specific organisms.

INTRODUCTION

Previous studies of the stomach contents of salmon in the Pacific Ocean have been based largely upon samples collected in the area of the Japanese high seas gillnet fishery in the northwestern Pacific (Nakai and Honjo, 1954; Allen and Aron, 1958; Andrievskaya, 1957; Ito, 1964). In the eastern Pacific the stomach contents of salmon caught only from coastal waters have been reported (Prakash, 1962; Reimers, 1964; LeBrasseur and Barner, MS, 1964). This paper describes the contents for various species including immature and maturing sockeye caught offshore in the northeastern Pacific. The stomach contents are further examined with respect to various oceanographic domains, as described by Dodimead et al. (1963) (Fig. 1), in which the fish were caught. These domains are areas in which various properties of the water such as structure, flow, heating, evaporation, and wind mixing are consistent.

MATERIAL AND METHODS

Four species of salmon, *Oncorhynchus gorbuscha* (pink), *O. kisutch* (coho), *O. keta* (chum), and *O. nerka* (sockeye), and one species of trout, *Salmo gairdnerii gairdnerii* (steelhead) were caught by surface gill nets fished overnight

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at the positions indicated in Fig. 1 during May and June 1958. The catches were frozen whole and stored. Subsequently, they were individually examined in a fresh-thawed condition. The age, sex, length, and weight were determined for salmon, but not for steelhead. These data together with observations of temperature, salinity, and other oceanographic features, have been published (Manzer and Neave, MS, 1959).

The fishing positions were assigned to domains as follows: stations 1, 3, and 5 to the Coastal Domain; station 2 to Alaskan Stream; stations 4, 7, and 8 to Central Subarctic; and station 6 to the Transitional Domain. The assignment of stations 2, 3, and 6 was somewhat arbitrary since they were in areas where domains overlapped.

The maturity of sockeye and chum salmon was determined according to the method suggested by Godfrey (1961). Maturity, as defined by this method is based on the development of the gonads at time of capture, and a salmon classed as immature in the spring might be reclassified as maturing later in the season. In referring to maturity of salmon the distinction made is between "maturing" fish, those which would spawn within the calendar year of capture, and "immature" fish, those which would probably not spawn until some subsequent year. Pink salmon were all classed as maturing since they were known

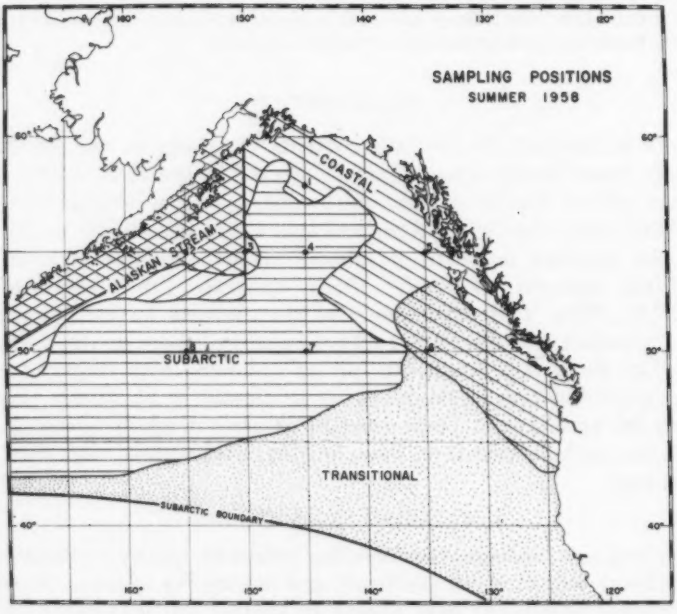


FIG. 1. Fishing positions and oceanographic Domains of northeastern Pacific Ocean, summer 1958.

to be in the 2nd year of their 2-year life cycle. Maturity of coho salmon and steelhead trout was not determined.

The weight of the stomach contents was determined by weighing the stomach once with the contents and again, with the contents removed, the difference being the weight of contents. The contents were identified within broad categories as: unidentifiable (due to advanced digestion), *Limacina* (pteropod), amphipod, copepod, euphausiid, squid, fish, and miscellaneous. Decapods and decapod larvae were the main forms included under miscellaneous. The weight of large organisms was determined directly; whereas, the weight of small organisms was estimated visually as a percentage of the total weight. Contents weighing less than 0.1 g were recorded as a trace. The stomach contents of 1216 fish (157 pink, 588 sockeye, 361 chum, 73 coho, and 37 steelhead trout) were examined. These data have been reported for each fish (LeBrasseur, 1965).

RESULTS

More than one generation of chum, coho, and sockeye salmon, and steelhead trout, are present together in the sea at any one time. Consequently, before attempting to investigate differences in stomach contents with respect to species or domains, stomach contents of immature and maturing chum and sockeye salmon were examined. Also, the stomach contents were examined from pink, chum, and sockeye salmon of different length. Data were not available for coho or steelhead for such comparisons.

Stomach Contents Related to Stage of Maturity and Size of Fish

Chum salmon from stations where both categories of maturity were represented in the samples by 10 or more individuals, i.e. stations 5, 6, 7, and 8, were grouped as immature or maturing fish. Sockeye, from stations 7 and 8, were similarly treated. The results of these comparisons of stomach contents of immature and maturing fish are set forth in Table I. Chi-square tests on the relative frequency of occurrence of various organisms indicate no significant difference in stomach contents of immature and maturing chum salmon, but a highly significant difference for these two categories of sockeye salmon. The latter is attributable to the greater incidence of squid in stomachs of maturing sockeye. On the basis of this comparison no further distinction was made with respect to maturity in chum salmon; however, immature and maturing sockeye were treated separately.

Salmon of different lengths were compared with respect to stomach contents. The samples treated were from domains having both a large sample size and wide range in length, i.e. pink and chum salmon from the Coastal Domain and sockeye from the Central Subarctic Domain. Figure 2 shows the percentage frequency of occurrence of various organisms in stomachs of salmon of different length. The stomach contents appear similar both for pink and chum salmon of different length. However, few small chum salmon were available for

TABLE I. Stomach contents of immature and maturing chum and sockeye salmon taken in May and June 1958 in the northeastern Pacific Ocean.

	Chum		Sockeye	
	Immature	Maturing	Immature	Maturing
Number:	121	109	114	49
Contents	*Frequency*	*Frequency*	*Frequency*	*Frequency*
Empty	9	10	33	8
Unidentifiable	98	88	11	1
Amphipod	84	78	72	25
Copepod	79	60	34	16
Euphausiid	10	9	3	0
Squid	2	4	9	28
Fish	1	0	0	0
Miscellaneous	2	8	2	5

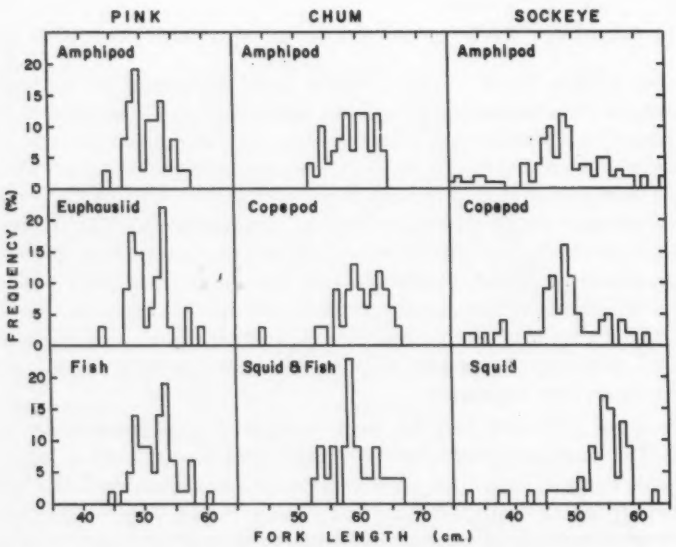


FIG. 2. Percentage frequency of occurrence of various organisms in pink, chum, and sockeye salmon of different length.

examination; only one chum was less than 50 cm. There is an obvious association between sockeye over 50 cm and the occurrence of squid. Thirty-eight of the 47 sockeye having squid in their stomachs were greater than 50 cm in length; whereas, of 111 sockeye with amphipods, only 37 were greater than 50 cm, and of 54 sockeye with copepods, only 14 were greater than 50 cm. Whether the difference found in stomach contents of sockeye of different length is related to maturity is uncertain as there is a considerable overlap in length between immature and maturing sockeye; also, as mentioned above, the criteria for maturity are not definitive. Examination for possible size effect within immature or maturing fish was not made.

The length of the sampled catch for each species within various domains is shown in Fig. 3. Of the species which were measured, most lengths fell

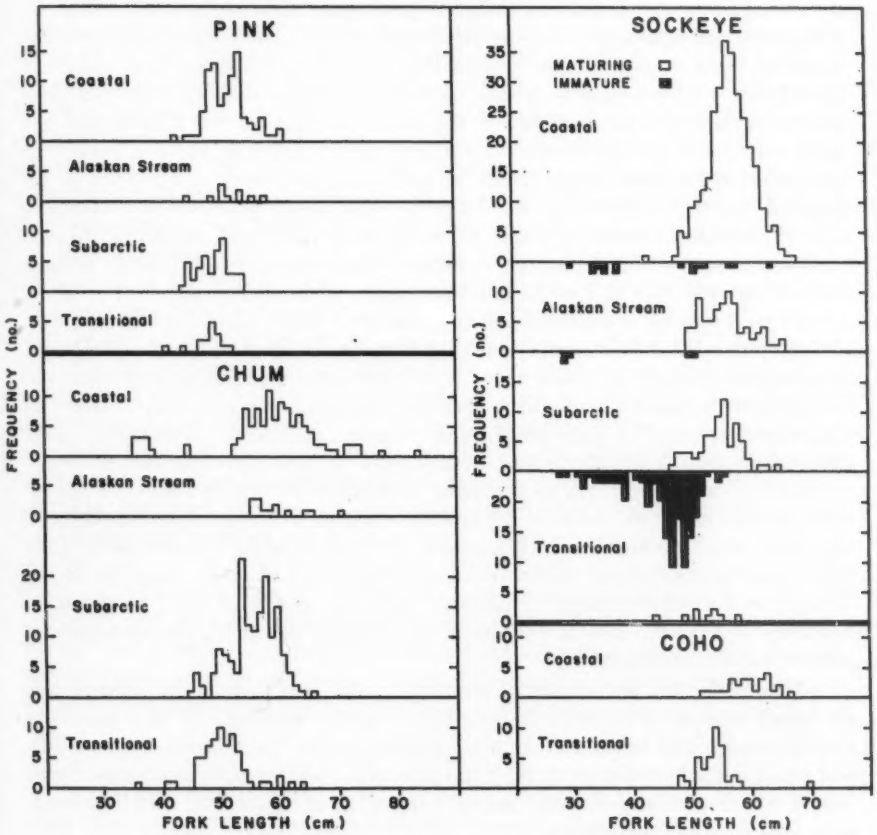


FIG. 3. Length-frequency of sampled catch of pink, chum, sockeye, and coho salmon in various ocean domains.

between 45 and 65 cm; immature sockeye providing most measurements under 45 cm. Except for immature sockeye which must be treated as a separate group, the length frequency distribution suggests that size differences as measured by length probably do not introduce a serious source of error in comparing the quantity and quality of contents between species in various ocean domains.

Stomach Contents Related to Species and Ocean Domains

Species were grouped into their respective domains and the stomach contents weighted to a constant sample size of 100 salmon. These data together with the percentage of salmon in which various organisms were present are reported in Table II. The total weight and estimated weight of various organisms making up the total have been taken from Table II to produce Fig. 4. Samples with less than five fish are not included in Fig. 4.

The principal difference in stomach contents of chum salmon in comparison with other species was the amount and frequency of occurrence of unidentifiable material. Only in the Alaskan Stream Domain did the frequency and weight of identifiable contents assume greater significance than unidentifiable contents; and here, the importance of fish in the stomach contents was overemphasized since only 2 of 14 chum sampled had fish in their stomachs. In fact, except for *Limacina*, which were in five chum, no organism was found in quantity in the stomach contents. However, elsewhere, the frequency with which amphipods and copepods occurred in trace amounts is notable and accounts in part for the low proportion of empty stomachs. Euphausiids or squid rarely showed up in more than two or three individual chums within a domain.

In pink salmon, most stomach contents were made up of euphausiids, fish, and amphipods. Other groups, such as *Limacina*, copepods, and squid, were occasionally present in more than trace amounts. One notable feature not immediately apparent from the percentage frequency data was the variety of organisms present in individual pink salmon stomachs; commonly, three different groups of organisms and, occasionally, up to six groups were found.

The stomach contents of maturing sockeye were predominantly euphausiids, squid, and fish. Smaller organisms, such as *Limacina*, amphipods, and copepods, were present in trace amounts in most samples. Occasionally, they were found in substantial quantities in sockeye stomachs, as for example, in the Transitional Domain where amphipods made up nearly a third of the total contents. Most of the stomach contents were readily identified. Empty stomachs were relatively common.

The most striking feature of stomach contents of immature sockeye was the small amount of material found. This is partly attributable to a generally smaller stomach as inferred from their smaller length. Amphipods, euphausiids, and squid contributed most to the total stomach contents. *Limacina* was absent from stomach contents of immature sockeye, while copepods and fish, if present, were only in small quantities.

Considerable quantities of euphausiids, squid, and fish were found in many coho stomachs. Small organisms were generally scarce, and almost all of the

material could be readily identified. Further, the proportion of empty stomachs was fairly high.

Only 37 steelhead trout were available for examination. Among their stomach contents, fish and squid were predominant. Amphipods were generally present, whereas, copepods and euphausiids were infrequently present, and never in more than trace amounts. *Limacina* was not found in steelhead stomachs.

The quantity of stomach contents varied widely between individuals of all species. Few fish had contents amounting to more than 1% of the total weight. Two salmon, a pink containing fish at station 5 and a sockeye containing squid at station 8, had contents whose weight exceeded 6% of the total body weight. However, most contents weighed between 1 and 10 g (LeBrasseur, 1965).

In general, representatives of each category of contents were present in some stomachs of each species. The only exceptions were *Limacina*, which were absent from stomachs of steelhead and immature sockeye, and amphipods, which were rarely found in coho stomachs. In contrast to the quality of contents, the quantity or amount of contents in stomachs showed striking differences between species. The amount of contents as shown in Table II and Fig. 4 is an average, being based upon both empty and full stomachs. If salmon with empty stomachs were omitted, species' differences as shown by the quantity of contents would be even further emphasized as, for example, in coho and sockeye salmon which had a relatively high proportion of empty stomachs.

The comparatively small difference found between species' stomach contents is in striking contrast to the differences in stomach contents found between domains. Two domains, the Alaskan Stream and Central Subarctic, were nearly monotypic as to the kind of organism making up the stomach contents. In the former, fish contributed the most to the total contents; in the latter, squid occupied an even more dominant position with respect to both per cent frequency and quantity. The stomach contents in the Coastal Domain were primarily fish and euphausiids. In the Transitional Domain, except for euphausiids, no two species contained entirely the same kinds of stomach contents. Besides exhibiting a marked dissimilarity in contents between species the Transitional Domain was noteworthy for the smallest quantity of stomach contents and the highest proportion of empty stomachs. If the comparison is made only between salmon having stomach contents, i.e. excluding empty stomachs, the quantity of stomach contents for the Transitional Domain remains the lowest. As might be expected, the Alaskan Stream and Coastal Domains were quite similar, the only real difference being the complete absence of copepods among stomach contents from the Alaskan Stream Domain.

DISCUSSION

Nakai and Honjo (1954) gave a preliminary report on the food of salmon taken close to the Aleutian Islands by the Japanese fishery in 1952. All species were grouped together and the food reported by percentage frequency of occurrence.

TABLE II. Stomach contents of salmonoids sampled in various oceanographic domains in the northeastern Pacific Ocean, May–June 1958.

Species	Domain	No.	Percentage empty	Total wt of identified contents	Organism													
					Amphipod		Copepod		Euphausiid		*Limacina*		Squid		Fish		Misc.		Unidentified	
					wt[a]	*%f*[b]	*wt*	*%f*	*wt*	*%f*	*wt*	*%f*	*wt*	*%f*	*wt*	*%f*	*wt*	*%f*	*wt*	*%f*
Pink	Coastal	94	4	775	74	43	9	21	194	45	87	24	4	9	270	48	137	28	130	29
	Alaskan stream	10	10	1770	10	50	0	0	40	20	tr	30	0	0	1720	50	tr	10	30	30
	Subarctic	47	15	260	38	55	tr[c]	11	tr	4	0	0	194	58	28	4	tr	2	tr	6
	Transition	16	31	255	94	69	12	47	118	20	19	33	0	0	6	7	6	6	19	7
Mature sockeye	Coastal	293	10	710	19	37	81	30	420	53	6	9	65	10	109	29	1	1	89	19
	Alaskan stream	79	15	819	78	49	0	0	170	27	42	16	41	13	488	46	tr	3	10	9
	Subarctic	71	14	1105	10	6	1	3	tr	9	0	0	980	52	49	1	56	1	0	0
	Transition	8	25	890	240	75	tr	25	630	25	0	0	0	0	0	0	20	12	35	25
Immature sockeye	Coastal	16	28	303	28	31	0	0	144	12	0	0	94	6	37	29	tr	29	150	44
	Alaskan stream	5	0	160	80	100	0	0	80	20	0	0	tr	20	0	0	0	0	0	0
	Subarctic	116	21	153	30	64	4	29	tr	3	0	0	114	9	3	3	2	2	1	1
	Transition	0																		
Chum	Coastal	116	3	127	11	50	2	30	20	15	tr	6	21	10	58	10	15	19	622	81
	Alaskan stream	14	0	1377	tr	29	0	0	tr	7	542	35	14	14	821	14	0	0	258	35

	Subarctic	160	10	19	1	70	tr	58	tr	9	0	0	14	6	4	2	tr	5	851	78
	Transition	71	3	30	tr	86	23	90	tr	8	0	0	tr	1	tr	1	7	4	400	86
Coho	Coastal	28	10	1426	tr	4	0	0	680	53	tr	7	183	7	510	46	54	32	tr	11
	Alaskan stream	0																		
	Subarctic	7	43	670	0	0	tr	14	0	0	0	0	670	43	0	0	0	0	tr	14
	Transition	38	37	470	tr	3	tr	8	128	13	5	13	275	29	30	8	32	8	5	5
Steelhead	Coastal	7	0	1427	128	57	0	0	0	0	0	0	0	0	1270	57	29	57	tr	1
	Alaskan stream	2																		
	Subarctic	20	10	1845	35	45	0	0	tr	5	0	0	1740	60	25	40	45	10	tr	10
	Transition	8	25	375	12	37	tr	25	tr	50	0	0	75	50	288	25	tr	12	62	12

[a]wt = weight of contents $\times \frac{100}{\text{number of samples}}$.

[b]%f = $\frac{Ni}{N} \times 100$. Ni = number of stomachs with a particular organism.

[c]tr = trace, where organisms totalled less than 0.1 g.

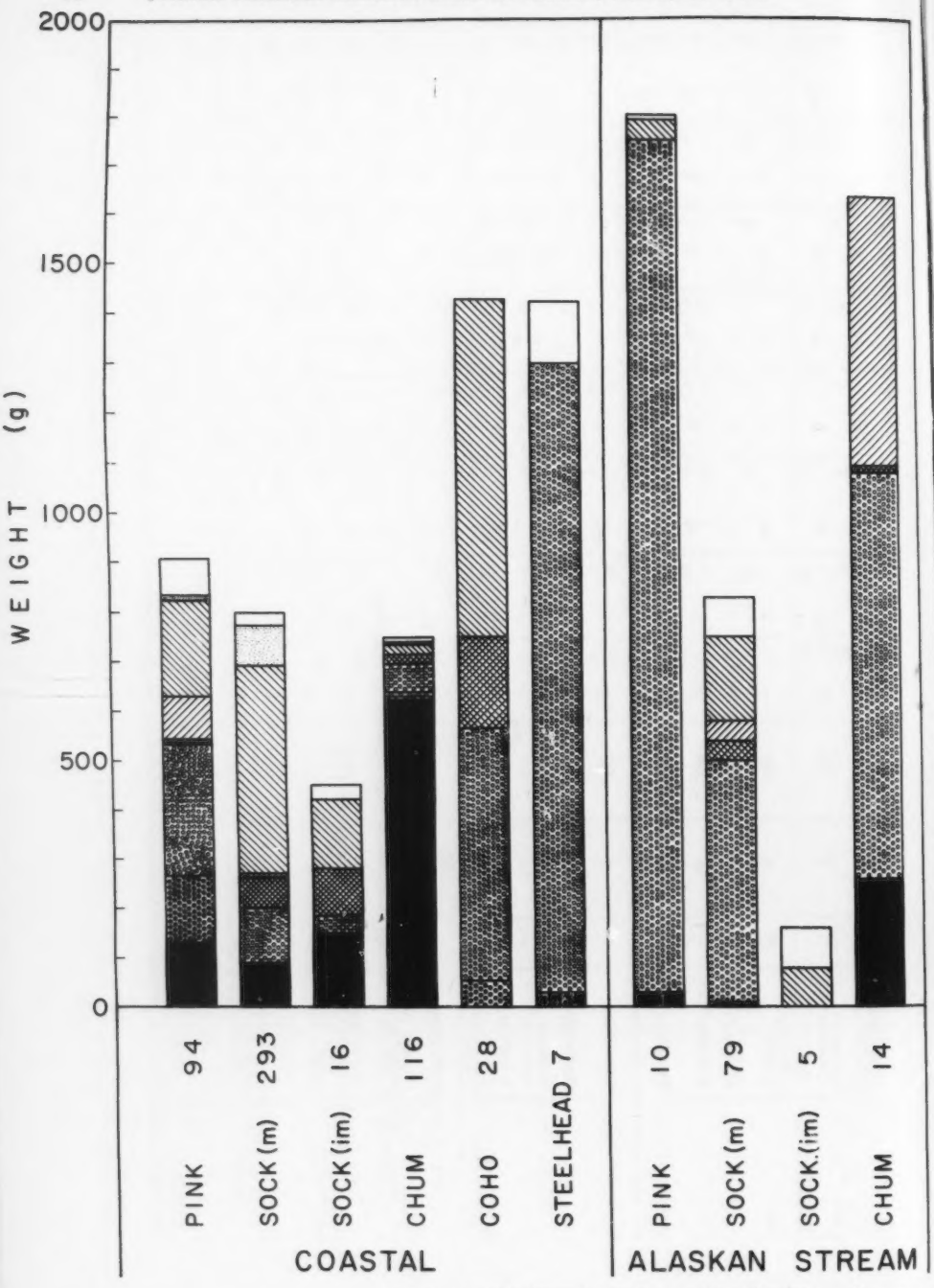
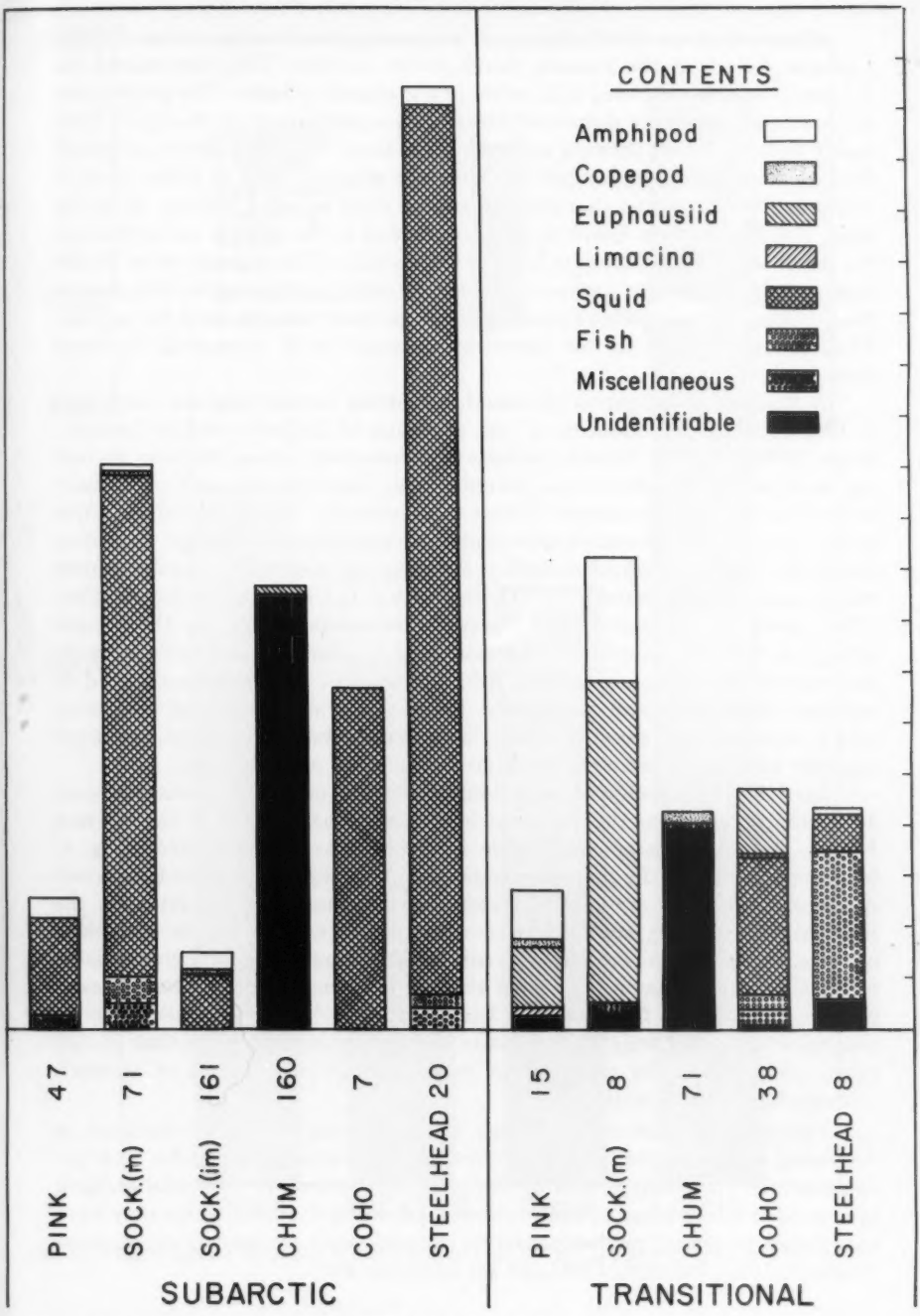


FIG. 4. Weight (grams) of salmon and steelhead stomach contents



per 100 fish in northeastern Pacific Ocean, 1958. (Data from Table II.)

Allen and Aron (1958) examined the stomachs of salmon taken by the Japanese fishery in the western North Pacific in 1955. They determined the volume of food by allowing it to settle in a graduate cylinder. The proportions of food organisms were estimated visually as a percentage of the total. They found little variation between stomach contents of the same species although food in chum stomachs tended to be further digested than in either pink or sockeye. They suggested that sockeye are the most selective feeders, chum the least. Amphipods were found to recur constantly in the salmon stomachs over the whole area of the Japanese fishery. Copepods and euphausiids were important mainly in oceanic waters. Contrary to what is reported in the eastern Pacific chum salmon tended to feed on the larger food, sockeye upon the smaller. The amount of food in the stomachs increased with increasing distances from land.

A Russian investigation of material collected from nearly the same area in 1955 reported the quantity of food as a ratio of the body weight (Andrievskaya, 1957). Over 60 different animals were identified among the food, including such items as radiolarians, oikopleurans, chaetognaths, and other soft-bodied forms. Using standard criteria for estimating the degree of maturity of the gonads, Andrievskaya was unable to associate any change in feeding habits with degree of sexual maturity. However, the degrees of sexual maturity which were defined, namely II–III, fall within the category which Godfrey (1961) designates as maturing; apparently, immature salmon, by the present definition, were not examined. The chief food of chum salmon was pteropods and euphausiids; of pink salmon, fish, euphausiids, and amphipods; and of sockeye, euphausiids and amphipods. Pink salmon stomachs contained more food than chum; and chum stomachs more than sockeye. In general, the mean quantity of stomach contents was higher than those reported here.

Ito (1964) has reported on a large collection of samples collected over an 8-year period from the Japanese high seas gillnet fishery in the western Pacific. Data from his appendix table 1 have been extracted for 1958 (Fig. 5) for comparison with the present results (Fig. 4). Steelhead were not reported. Although these data are from a region which includes several domains, the similarity of stomach contents from western and eastern Pacific salmon is obvious, even to the presence of considerable quantities of unidentifiable material in chum salmon. The most striking difference is the greater amount of stomach contents from western Pacific salmon. A most interesting result was the finding that weight of contents was greater in even years than in odd years, and, during the most recent years, the average weight of stomach contents has been increasing.

The stomach contents of oceanic steelhead were previously reported as consisting of fish, squid, and trace amounts of crustaceans (Taylor and LeBrasseur, 1957). Other records of the stomach contents of coho and chinook salmon have been limited to observations made along the coast where they have been found to consist predominantly of fish and small amounts of crustaceans (Prakash, 1962; Reimers, 1964).

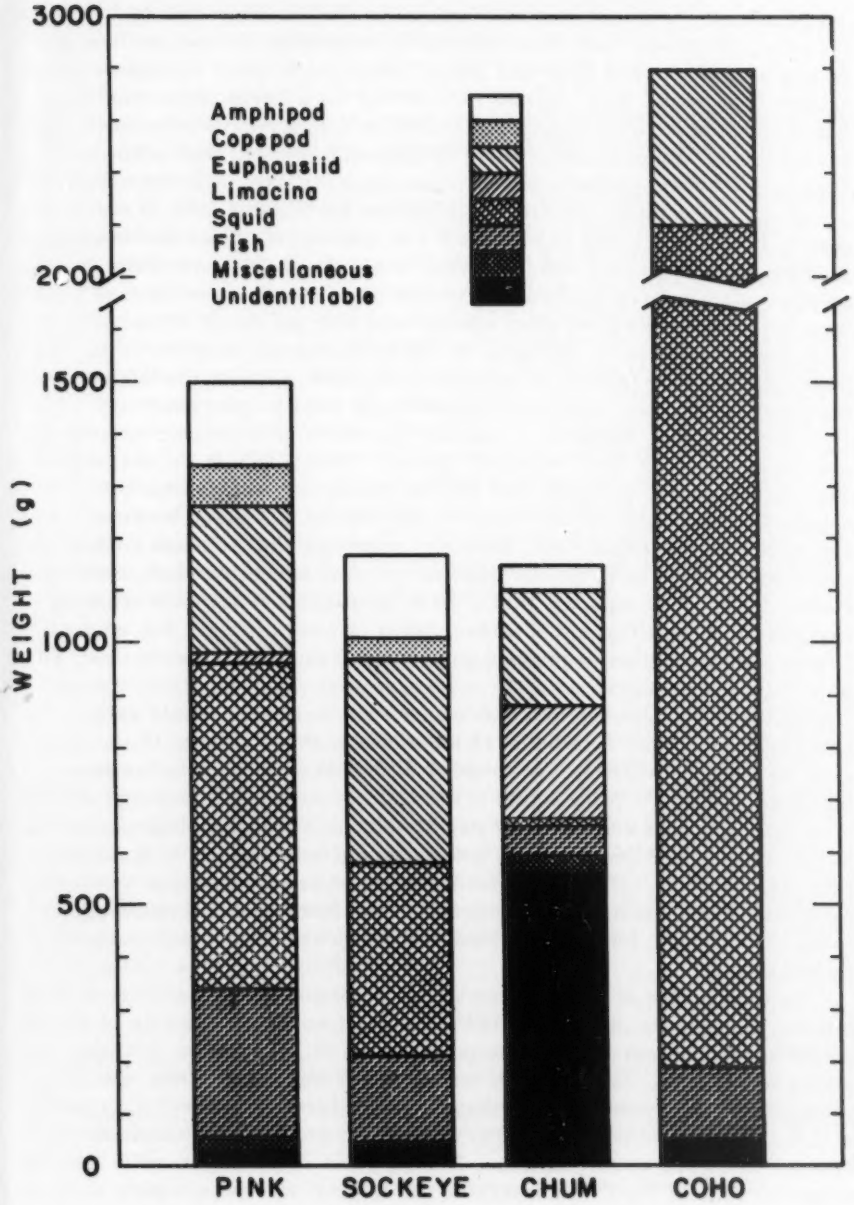


FIG. 5. Weight (grams) of salmon stomach contents per 100 fish in northwestern Pacific Ocean, 1958. (Data from Ito, 1964.)

The term "stomach contents" has been used in preference to "food" in reporting the present data since there is no knowledge of what, or, how much of what is present had been assimilated immediately prior to capture of the salmon. The term "food" should be reserved for all organisms eaten by the fish. The list of stomach contents identified by Andrievskaya includes 10 times the number of organisms contained in the present report and there is no certainty that hers is a complete list. Certainly, size, quantity, and structure of different organisms will influence the rate at which they are digested, and, of course, the ease with which they may be identified. The quantity of unidentifiable material in chum salmon stomach contents serves to emphasize this point, since one can only speculate as to its original components. Possibly, the same kinds of organisms found in stomachs of other species were also present in chum stomachs. The presence of trace amounts of these organisms supports this view; however, organisms present in trace amounts could represent the last stages of digestion or they may have been ingested just prior to capture.

Chum salmon represent a special case both with respect to contents and morphology of their stomach (Anon., 1964); and, it is not unlikely that their digestive processes and feeding habits also differ. Motohiro (1962) compared the activity of the digestive enzymes of chum and sockeye. From the results of this experiment, Motohiro suggested that stomach contents of chum salmon were more rapidly metabolized than in sockeye. LeBrasseur and Barner (MS, 1964) reported that a large proportion of stomach contents of juvenile (underyearling) chum salmon taken in coastal waters was unidentifiable in contrast to that of juvenile pink salmon caught at the same time. This observation is consistent with the suggestion that rapid digestion of stomach contents is a characteristic of chum salmon which, in turn, would explain the small amount of fresh material in chum stomachs. Alternatively, chum salmon may feed more extensively upon readily digestible organisms. LeBrasseur and Stephens (1965) used swine pepsin to determine the relative rate of degradation of marine organisms known to be eaten by salmon. The rate of degradation was in the order (1) fish (myctophids, hexagrammids, salmonids); (2) squid (gonatids); (3) copepods; (4) euphausiids. A similar pattern for digestion of contents could take place in salmon, which, together with differences in organisms ingested, could also account for the small amount of identified stomach contents in chum salmon.

In the absence of direct observations of salmon feeding activity it is of interest to speculate about the time of feeding from the percentage of empty stomachs. The gillnet fishing was generally of 10 hr duration, covering the period of darkness. Salmon could be caught at any time during the 10 hr. Once caught, they probably die, although, again, there are no direct observational data. Feeding and passage of food through the gut ceases, while digestion of stomach contents would continue. A high ratio of stomachs with food to stomachs without food would suggest the feeding act was complete or near completion, whereas, a low ratio would suggest feeding was just commencing or about to commence. Under this simple scheme chum salmon are apparently

caught first subsequent to feeding, and sockeye and coho last, in the act of feeding. One may postulate that chum salmon tend to feed early in the evening as the nets were being set while other salmon species have a tendency to feed in early morning, closer to the time the nets are being retrieved. A suitable test for the above conjectures would be to fish for short intervals through several days and examine the proportions of the species caught and their stomach contents.

It is evident that a small change in the availability of some organisms could produce a significant change in the quantity of contents found in stomachs. For example, in terms of biomass one squid or fish represents many hundreds or even thousands of smaller organisms such as amphipods. Obviously, it would be more efficient to feed exclusively on large prey if they were available: and, as has been shown, fish and squid, as exemplified by their presence mainly in stomachs from the Coastal and Central Subarctic Domains, respectively, were not equally available in all areas. Furthermore, Ito's (1964) observations in the western Pacific of an even-year cycle for squid and a corresponding cycle in the quantity of food in stomachs suggests there are annual as well as regional differences in the availability of prey. This observation together with the possible association of specific organisms with particular domains or areas suggests that a study of salmon growth and year-to-year changes in their total weight might be most rapidly advanced by investigation of one or two major organisms rather than the entire spectrum of potential prey.

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Inter-Species Relationships Within the Genus *Oncorhynchus* Based on Biochemical Systematics[1]

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ABSTRACT

The species specific muscle myogens of *Salmo gairdnerii*, *Oncorhynchus masou*, *O. masou ishikawae*, *O. kisutch*, *O. tshawytscha*, *O. keta*, *O. nerka*, and *O. gorbuscha* are compared by starch gel electrophoresis. Plasma proteins of these same species are also examined by polyacrylamide disc electrophoresis. The range of usefulness of muscle myogens in species identification, and equally significantly, their value in establishing phylogenetic relationships of closely related groups, as the genus *Oncorhynchus*, are discussed. The myogen patterns of *O. keta* and *O. gorbuscha* from the Asiatic and North American coasts were found to be identical, further supporting the concept of absolute species specificity of these patterns.

INTRODUCTION

In an examination of over 6000 fishes covering about 100 marine and freshwater species (Tsuyuki and Roberts, 1963, 1965; Tsuyuki et al., 1965a) electrophoretic patterns of muscle myogens and blood proteins have proven to be species specific. During these studies it became evident that protein patterns, particularly the muscle myogens, are virtually unaffected by factors other than genetic. It is species specific and extremely invariable within a given species so that an analysis from a single specimen gives reliable results. The possible variations in the mobilities of the numerous protein zones are very great, increasing the value of protein patterns as a single criterion in the identification of a large number of species. Through the study of the interspecific relationships within a number of genera, for example, *Salmo*, *Salvelinus*, *Lepomis*, *Sebastodes*, and particularly *Oncorhynchus*, it was observed that the degree of similarity of muscle myogen patterns, and hence the individual zones, appear to be related directly to the extent of closeness in phylogenetic relationship established by other taxonomic methods. If the myogen patterns were not so related, their usefulness in phylogenetic studies would be limited, although species specificity might still be maintained. When the patterns between two individuals become indistinguishable it is also difficult to segregate the two by any other means. Under these circumstances, electrophoretic analyses of blood proteins may provide additional identifying properties.

So far only three species, *Anoplopoma fimbria* (Tsuyuki et al., 1965a), *Stizostedion vitreum* (unpublished data), and *Catostomus catostomus* (unpublished data), were encountered in which the systematic variations in muscle

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myogen patterns were not supported by known morphological differences. The protein patterns in these examples varied in a way expected of known hybrids (Tsuyuki and Roberts, 1965; Manwell et al., 1963), that is, the phenomena of additivity of parental protein zones in the hybrid were observed.

In all specimens of *S. vitreum* and *C. catostomus* from Great Slave Lake only one type of myogen pattern was observed and significantly the other "parental" as well as the hybrid types were absent. In contrast those specimens from the Great Lakes area displayed both parental as well as the hybrid types of patterns. It seems improbable that these geographically linked systematic variations are examples of breakdown in myogen specificity. More likely, the differences in their genetic history are reflected in these protein patterns. Instances of morphological differences not supported by readily recognizable differences in muscle myogen patterns were observed in *Sebastodes ciliatus* and *Sebastodes polyspinis* (Tsuyuki et al., 1965a). In these two rockfishes, the multiple hemoglobins as well as the plasma protein (unpublished data) patterns were virtually identical. Because of the critical nature of these analyses in deciding the biochemical uniformity of these two species the blood protein separations are being repeated.

It is not intended here to review the interrelationships of salmon, for an excellent guide to this literature is available (Wilimovsky and Freihofer, 1957), but to review evidences based on biochemical systematics.

In the present report the results of electrophoretic studies have been used to show the interspecific relationship within the genus *Oncorhynchus* as well as the relationship of this genus with that of the *Salmo*.

MATERIALS AND METHODS

Muscle and blood plasma samples of *Oncorhynchus masou*, *O. masou ishikawae*, *O. keta*, and *O. gorbuscha* were obtained from Hokkaido, Japan. *Salmo gairdnerii* was obtained from the Sun Valley Trout Farm in Port Coquitlam, B. C., and the North American samples of *O. keta* and *O. gorbuscha* from the Fraser and Thompson Rivers, respectively, in British Columbia. *Oncorhynchus tshawytscha* and *O. kisutch* were obtained from Spring Creek and Green River hatcheries, respectively, in the State of Washington.

The methods used for the preparation of muscle extracts and for starch gel electrophoresis have been described previously (Tsuyuki et al., 1962, 1965a). Plasma proteins were analysed by the Canalco Model 12 polyacrylamide disc electrophoretic technique.

RESULTS AND DISCUSSION

The phylogenetic interrelationship of the species belonging to the genus *Oncorhynchus* is examined on the basis of information derived from the results of studies in biochemical systematics. The muscle myogen patterns of *O. masou* and its landlocked variety, *O. masou ishikawae*, *O. kisutch*, *O. tshawytscha*, *O. keta*, *O. nerka*, and *O. gorbuscha* are compared, on the basis of a simultaneous

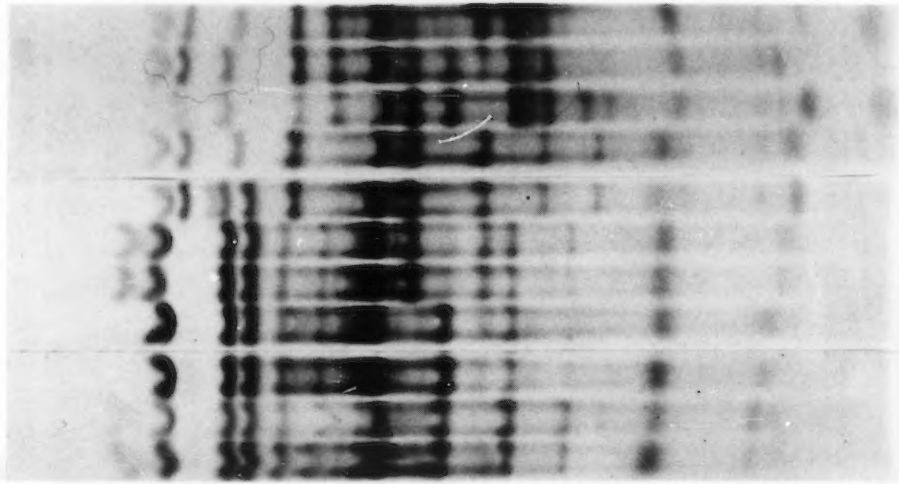

FIG. 1. A comparison of starch gel electropherograms of the muscle myogens of the genus *Salmo* and six species from the genus *Oncorhynchus*. In this and subsequent illustrations, the anode is to the right and the cathode to the left.

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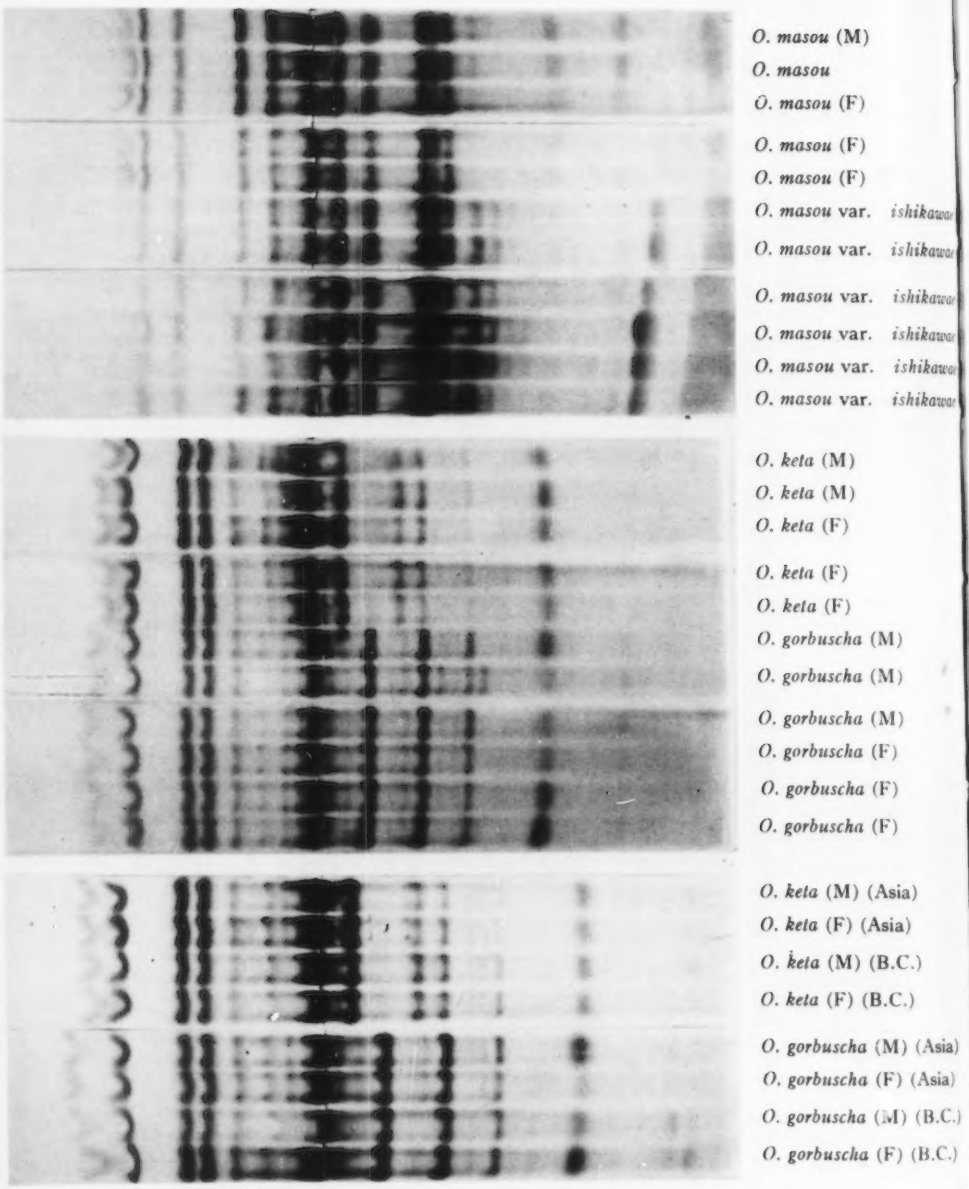

FIG. 2. Starch gel electropherograms of the muscle myogens of *O. masou* and *O. masou ishikawae* (A); *O. keta* and *O. gorbuscha* from Hokkaido, Japan (B). In (C), Asiatic and North American samples of *O. keta* and *O. gorbuscha* are compared.

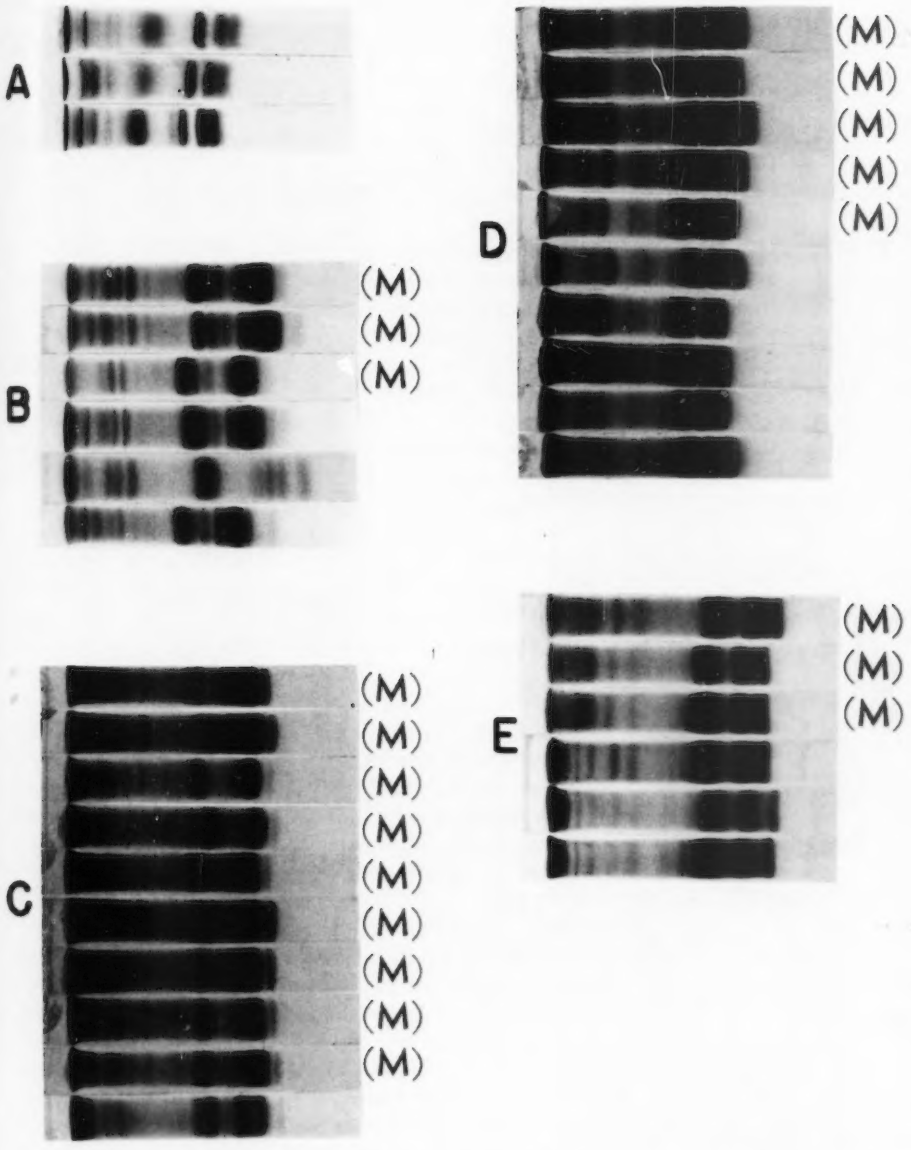

FIG. 3. The comparative disc electrophoretic patterns of the plasma proteins of *Salmo gairdnerii*, (A); *O. masou* from Hokkaido, Japan, (B); *O. kisutch* from the Green River hatchery in Washington, (C); *O. tshawytscha* from the Spring Creek National hatchery in Washington, (D); *O. keta* from Hokkaido, Japan, (E); (*continued on next page*).

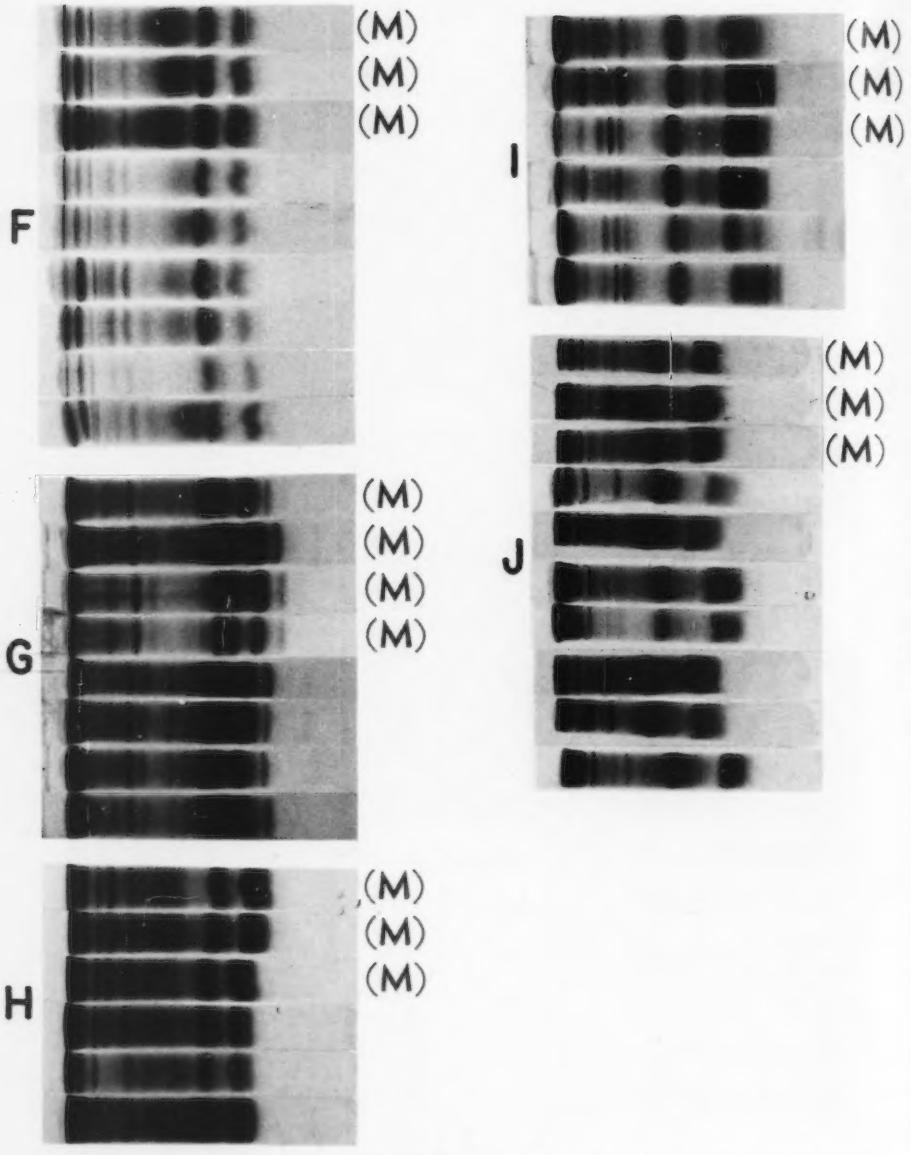

FIG. 3 (*Concluded*). *O. keta* from the Chehalis River, B. C., (F); *O. keta* from the lower Fraser River, B. C., (G); *O. nerka* from the Pitt River, B. C., (H); *O. gorbuscha* from Hokkaido, Japan, (I); *O. gorbuscha* from the Thompson River, B. C., (J). Male specimens are designated by "M"; all others are females.

starch gel electrophoretic analysis, to each other and to *Salmo gairdnerii* used here as a representative of the genus *Salmo* (Fig. 1). The species are arranged in the order of their phylogenetic relationship to the genus *Salmo*, on the basis of the similarities in their protein patterns, as well as of the manner in which a specific enzyme protein, enolase (Tsuyuki and Wold, 1964) has evolved. This order of arrangement of *O. kisutch*, *O. tshawytscha*, *O. keta*, *O. nerka*, *O. gorbuscha* agrees with previous studies (Tsuyuki and Roberts, 1963) and with those of Hikita (1962) with the exception of the positions of *O. gorbuscha* and *O. nerka*.

The positions of these two species could as well be reversed for the most cathodic zone of *O. keta* shown is separated into two while in a previous study (Tsuyuki et al., 1965a) this zone appeared as one. Further studies are in progress to clarify this discrepancy. The position of *O. keta* appears to lie directly between *O. tshawytscha* and *O. nerka*. Other differences to this order are found in the morphological studies of Clemens (1953) and the serological studies of Hourston (MS, 1949).

It appears from the myogen patterns that *O. masou* is phylogenetically much closer to the *Salmo* than to the *O. kisutch–O. tshawytscha* group and certainly than to the *O. keta–O. nerka–O. gorbuscha* group. The cluster of intense zones on the anodic side, typically present in all species of *Salmo* examined and absent in five other species of *Oncorhynchus*, are also very much in evidence in the *O. masou* and its landlocked variety, tying this species even closer to the *Salmo*. It is interesting to recall here that the relative position of the muscle myogen of *O. masou* between those of *Salmo* and *O. kisutch* was predicted in a previous study (Tsuyuki and Roberts, 1963) on the basis of other phylogenetic considerations (Neave, 1958; Hikita, 1962). This Asiatic species is generally believed to have been the first to evolve from the *Salmo* (Neave, 1958), and of existing species the most closely related (Regan, 1920) ecologically and physiologically (Tanaka, 1963) and by the present study, biochemically. However, if *O. rhodurus*, a completely freshwater form, occupies a position of intermediacy between *O. masou* and *Salmo* (Hikita, 1962), it would be interesting to see whether the muscle myogen pattern of this species is closer still to the *Salmo*.

In feeding experiments, Fujita (1933) reported that 37 out of 103 fry obtained from *O. masou* spawners became yamame (landlocked). This tendency has also been reported by Saguri (1936). Although it has been claimed that without exception all anadromous *O. masou* die after their first spawning (Ono, 1933; Sano, 1947), in some feeding experiments yamame have spawned successfully two or more times (Tanaka, 1963). In this respect *O. masou* by virtue of the behaviour of its landlocked form, *ishikawae*, is closely related to the genus *Salmo*, as can be seen clearly from their muscle myogen patterns. *Oncorhynchus masou ishikawae* exist in many different forms depending on the freshwater systems they inhabit and has been described as a new species, *O. ishikawae* (Jordan, 1925), but has since been treated synonymously with the landlocked form of *O. masou* (Hikita, 1962). Certainly, the six specimens

(four male, two female) of yamame we have examined possess a muscle myogen pattern distinctly different from the five specimens (two male and three female) of anadromous *O. masou* compared simultaneously (Fig. 2A). This order of difference in the protein patterns is what might be expected at the species level (Fig. 1), at which it has once been placed (Jordan, 1925), and most likely would not result unless this particular population had been isolated from the anadromous variety for a period of time sufficient to develop genetically distinctive characteristics. The kokanee, a "landlocked" variety of *O. nerka*, captured in freshwater system which is freely accessible to the ocean, has not yet developed protein patterns distinctly different from its anadromous ancestors. The effect of the age of the yamame on its myogen pattern has not been taken into account. The average length of the six specimens was 14 cm (average weight, 30 g) while the average length of the *O. masou* was 48.2 cm. However, previous studies on Pacific salmon have shown that the variations in the myogen patterns of the young are still within the specificity range of that species and readily distinguishable from other members of this genus (Tsuyuki et al., 1965b).

Five specimens of *O. keta* (average length 63 cm) (two males and three females) and six *O. gorbuscha* (average length 47.4 cm) (three males and three females) from Hokkaido are compared in Fig. 2B. The muscle myogen patterns are reproducible, independent of differences in sex, and completely species specific. One *O. gorbuscha* male sample displayed an extra minor anodic zone, but clearly all other zones are species specific and should not pose any problems in identification. The muscle myogens of these Asiatic samples were compared by a simultaneous electrophoretic run to adults of the species from the North American coast (Fig. 2C). There are virtually no outstanding differences between North American and Asiatic specimens of *O. keta* and *O. gorbuscha*. A minor zone just to the cathode side of the origin appearing in Asiatic samples of both species is not a real difference, for freshly-captured North American samples also possessed this zone which disappears upon extended storage. It is remarkable that the two species homing to such widely separated geographical areas have identical patterns, supporting further the absolute species specificity of muscle myogen patterns.

The plasma proteins of rainbow trout and six species of *Oncorhynchus* are shown in Fig. 3. A number of individuals of each species of salmon is presented, primarily to point out the extent of variations, though less than freshwater species, that can be expected in agreement with the studies of others reviewed by Booke (1964). Although species specificity is maintained it is not nearly as self-evident as the muscle myogen pattern (compare with Fig. 1). Phylogenetic interrelationships could be established only with difficulty from the plasma protein patterns as was also the case for many other genera examined. Most North American salmon samples were examined within 24 hr from the death of the fish by starch gel electrophoresis which was found to give inadequate resolution under the conditions used for the separation of human sera. Consequently, the remainder of the plasma samples were stored

at –30 C for a period as long as 12 months before the Canalco Model 12 disc electrophoretic unit could be assembled and used for more effective resolutions. Frozen storage for a period of 1 week did not alter greatly the original pattern of freshly-obtained plasma proteins of rainbow trout (Fig. 3A). Subsequently, it became evident that prolonged frozen storage did not affect the patterns drastically. However, repeated freezing and thawing resulted in increasing precipitation, probably of the globulins, for this region of the plasma patterns lost most of the minor zones characteristically present in fresh samples. These changes are shown in the disc patterns of North American *O. keta*. Figure 3F shows the usual plasma patterns of *O. keta* while in Fig. 3G the samples were repeatedly frozen and thawed.

Although the exact period is not known, the *O. masou* (Fig. 3B) and the Asiatic samples of *O. keta* (Fig. 3E) and *O. gorbuscha* (Fig. 3I) have probably been frozen for 2–3 months and the patterns shown do not indicate extensive changes in the globulin region. Unfortunately, fresh plasma samples are not available for comparison. The Asiatic *O. keta* and *O. gorbuscha* are compared with their North American counterparts (see Fig. 3E, F, I, and J). Considerable differences were observed in the plasma patterns of these two species from the two geographical areas. These differences may have resulted from frozen storage and consequently final decision must be reserved until a direct electrophoretic comparison could be made on fresh plasma samples. Patterns of North American *O. nerka* (Fig. 3H) are included for comparison with the *O. keta* and *O. gorbuscha*.

The plasma proteins of the *O. masou* (Fig. 3B), *O. kisutch* (Fig. 3C), and *O. tshawytscha* (Fig. 3D) are compared since those three species are considered to be closely related (Hikita, 1962). Species specificity is maintained but phylogenetic relationship of this group is practically indistinguishable from the *O. keta–nerka–gorbuscha* group on the basis of plasma protein patterns.

In some species, the female plasma patterns are characterized by a heavy zone just below the origin and in others there is no systematic difference. The North American *O. keta* in Fig. 3F do not, while the repeatedly frozen and thawed samples in Fig. 3G do show sexual differences. As all the North American samples shown here were captured within a few days of their spawning times, it is apparent that the extensive development of the gonads of either sex do not greatly influence the plasma protein patterns at this time of sampling.

Finally, it must be pointed out at this time that many of the faint protein zones to which references are made in this and in previous publications were frequently unavoidably lost upon photographic reproduction. However, all the zones considered were clearly visible in the original starch gels. Other means of presentation of the patterns, such as densitometer tracings, have been considered and tried (Vanstone et al., 1964), but it was felt that the disadvantages outweighed the advantages.

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Vertical Migration by Demersal Fish in the Northwest Atlantic[1]

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ABSTRACT

Observations on vertical patterns of migration were made on the Nova Scotian and Gulf of St. Lawrence fishing banks. The commercially important demersal species, cod, haddock, and redfish, were usually closely associated with bottom by day and moved off after dark. While this is the general pattern, variations occurred. Thus, on cloudy or dull days, redfish may remain in mid water. Cod did not always return to the seabed by day. Haddock, usually more closely associated with bottom than the other species, sometimes moved considerable distances from the seabed. Generally, concentrations of fish on bottom by day dispersed in mid water.

In accord with vertical patterns of migration, bottom trawl catches were generally the lower by night. There was some suggestion of seasonal changes in the relation between day and night catches of cod and haddock. A suggestion of diurnal variation in size composition was found for haddock and yellowtail. More small haddock were taken by day than by night. Catches of large haddock tended to be the larger at night. Small yellowtail were the more abundant in night tows. No significant diurnal difference was found for large fish.

INTRODUCTION

Customarily, demersal fish are captured by otter trawls which are towed along the seabed. Particularly since the development of echo sounders it has been evident that fish spend much of their time in intermediate layers rather than on bottom. This is reflected in lower catches. As a general rule, fish remain on bottom by day and move into upper layers with the onset of darkness. In the eastern North Atlantic, investigators have expended considerable effort to establish diurnal behaviour patterns for commercially important species (Hickling, 1935; Lucas, 1936; Schmidt, 1955; Jones, 1956; Bagenal, 1958; Woodhead, 1960, 1961, 1964; Parrish et al., 1964; and others). These studies have revealed not only general patterns of vertical migration but also deviations. Thus, Ellis (1956), Trout (1957), Sundnes (1964), and Woodhead (1964) observed fish off bottom by day and by night under particular environmental conditions. Some have observed behavioural changes in shoals on and off bottom (Konstantinov and Scherbino, 1958; Sundnes, 1964). Dispersion in mid water reduces the practicability of mid-water fishing whereas the continuance of compact shoals enhances the possibility. Others have measured vertical distances migrated (Cushing, in Richardson et al., 1959; Trout, 1962). Diurnal changes in size composition have attracted the attention

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of still others (Jones, 1956; Woodhead, 1963; Boerema, 1964; Parrish et al., 1964).

While many facets have received consideration in the eastern North Atlantic fishery, information in the western North Atlantic is particularly scarce, the work of Brunel (1964) and Templeman (1959) being notable exceptions. Between 1958 and 1964, the Fisheries Research Board's research vessels, *A. T. Cameron* and *Harengus*, have collected information on vertical migration on the Nova Scotian and Gulf of St. Lawrence fishing banks. Typical patterns of migration were recorded for cod, haddock, redfish, and flatfish by echo sounder. Further, these records were analysed to measure distance of migration and to study dispersion of concentrations off bottom. Day and night catches by bottom trawl were determined and related to patterns of vertical migration. The suggestion that it is predominantly fish of one size that migrate (Woodhead, 1964) prompted a comparison of size composition by day and night.

MATERIALS AND METHODS

Much of the study is based on research-vessel surveys conducted from 1958 to 1964 with the CGS *A. T. Cameron* and MV *Harengus*. Both vessels are equipped with echo-sounding equipment which is operated almost continuously during a cruise. A Simrad echo sounder (model 510-5) with moist paper and "white line," operating on 38 kc, was used on the *A. T. Cameron* along with a 30-kc Electroacustic fishlupe (model Arcturus). The *Harengus* is equipped with a dry paper Electroacustic sounder (model Atair) with white line as well as a fishlupe. Both operate at 30 kc.

Capture of fish was by otter trawl. All tows by the *A. T. Cameron* were made with a standard 41-5 manila trawl. The *Harengus* is equipped with a #36 manila otter trawl. Jean (1964) gives dimensions for both nets. On both vessels the codend was either covered or lined with 25–30 mm mesh netting to reduce escapement by small fish. The length of tow was standardized as 30 min on both vessels. Whenever practicable, all fish were measured. However, catches greater than 30 baskets (or about 1200 kg) were sampled.

To compare catches by day and by night, the time of sunrise and sunset for each day and each area was calculated by the method outlined in Canadian Almanac and Directory (1963 edition). All hauls made between sunrise and sunset, except those made within the hour of sunrise and sunset, were taken as day tows. Sunrise and sunset hauls were omitted in this analysis.

The area investigated extends from Orphan and Miscou Banks in the Gulf of St. Lawrence southward along the east coast of Nova Scotia to Browns Bank off the southern tip of Nova Scotia. Most of the *A. T. Cameron* cruises were made in winter and spring months. Indeed, of 25 groundfish cruises on the *A. T. Cameron*, all but three were made between January and May. Two were made in October and one in July. Most of the cruises were designed to study distribution; however, many observations were made on regularity of vertical migrations, distances moved, diurnal variations in catch and size

composition. The *Harengus* made one cruise in September, one in November, and one in October specifically to compare day and night catches.

Questionnaires and personal interviews with captains of commercial fishing vessels permitted, in a general way, a wider coverage of vertical migration patterns for all fishing areas throughout the year. More detailed data were collected by trained Fisheries Research Board observers aboard commercial vessels. In these cases, an estimate of the weight per tow by species was recorded along with the time, depth, and area. These are used primarily for areas and seasons not covered by our research vessels.

Identification of echo traces was based on otter-trawl catches. With traces on bottom trawl catches were used to establish species in an area. Usually only one species occurred in abundance. Subsequent observation of traces off bottom was related to species distribution. In mid water echoes from gadoids are readily separable from redfish and flatfish, the fish commonly taken in abundance. Among the gadoids separation of cod and haddock echoes is extremely difficult. Fortunately, research cruises were operated in areas where the two species seldom occurred together in appreciable quantities. For example, in cruises to the Gulf of St. Lawrence region, cod and redfish were the dominant species (Jean, 1964). Similarly on cruises to Sable Island and westward, haddock predominated (McCracken, 1964).

Interpretation of echograms is not usually difficult. A simplified drawing in Fig. 1A illustrates the salient features, while Fig. 1B is an actual sounding. The depth of water is the distance between the transducer, located on the underside of the ship's hull, and bottom. With no fish on the seabed, the bottom line remains constant in thickness despite uneven bottom. However, with fish on bottom, the line thickens in the manner illustrated. The "white line" effect, simply a blocking out of a portion of the strong seabed echo, is useful in separating fish on bottom from irregularities in topography. Off bottom, echo traces of fish are normally obvious as in the figure.

RESULTS

Vertical Migration

COD, *GADUS MORHUA* LINNAEUS

Echo soundings from the Gulf of St. Lawrence and Nova Scotian fishing banks suggest that cod generally move off bottom by night and return to the seabed at daybreak. Only occasionally have echoes been observed off bottom by day. Figures 2 and 3 illustrate typical nocturnal vertical migrations. In Fig. 2, traces can be observed off bottom at dusk (1700 AST), well off bottom at night (2300 AST), returning to bottom after daybreak (0815 AST), and on bottom by day (0923 AST). The figure illustrates further the breakdown of a school off bottom by night and its reformation on bottom by day. Perhaps a better illustration of dispersion in mid water is shown in Fig. 3.

Not infrequently, traces have been observed more than 110 m off bottom. A summary of the vertical movements of cod by day and by night is given in

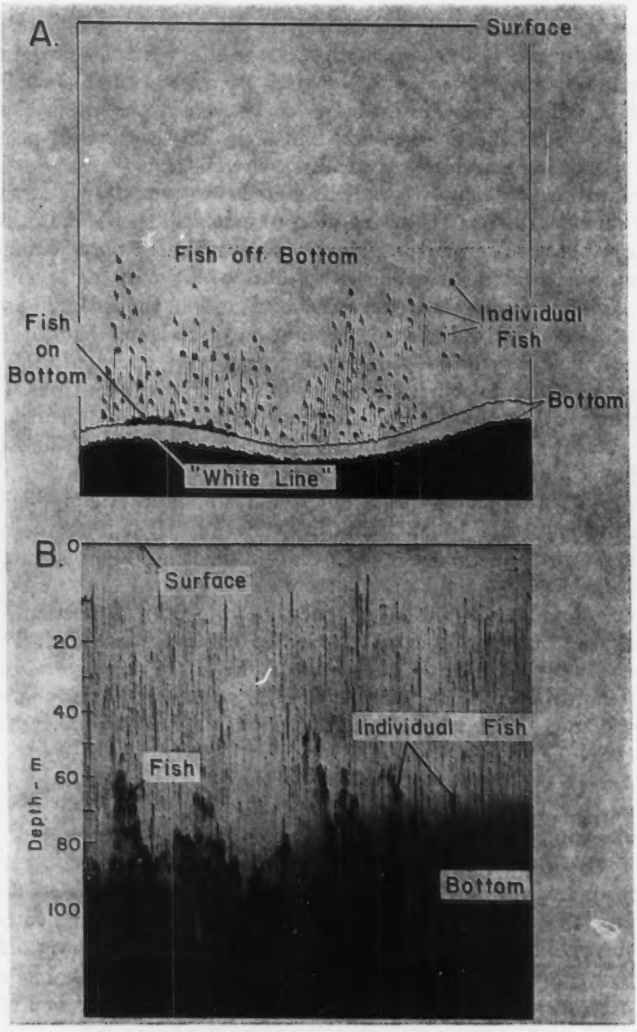

FIG. 1. **A.** Diagram of echogram. Fish on the seabed are identified by thickening of bottom line. Off bottom, individual fish appear as distinct marks. **B.** Actual echogram illustrating fish on and off bottom.

FIG. 2. Traces of cod on bottom during the day (0815 and 0923 AST) and off bottom at night (1700 and 2300 AST). The surface to bottom depth ranged from 165 to 210 m.

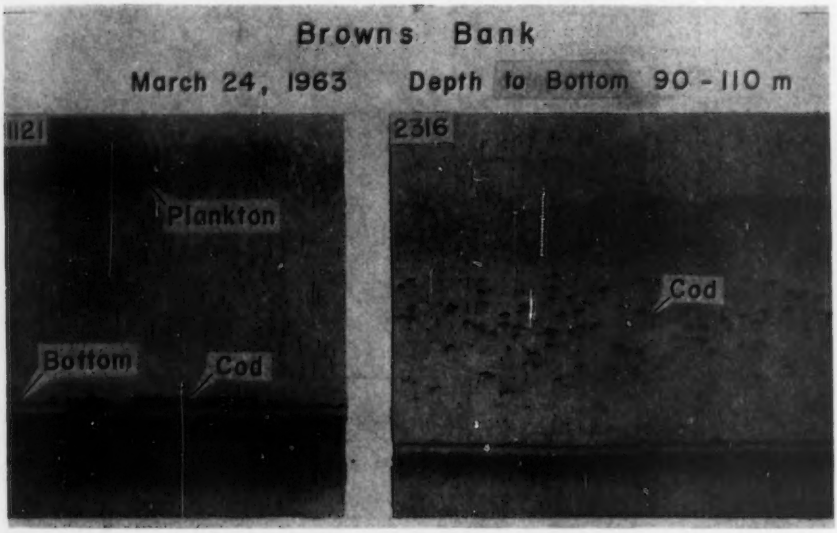

FIG. 3. Traces of cod on bottom during the day (1121 AST) and off bottom at night (2316 AST). The depth at time 1121 is about 102 m while that at time 2316 is 119 m. The smaller traces near the surface are believed to be from plankton.

Fig. 4. The data were collected on the Gulf of St. Lawrence and Nova Scotian banks between 1960 and 1963. Each point in the figure represents a mean distance from bottom for a group of echoes. For example, echo traces might be recorded on bottom and as far as 10 m off bottom. Providing the distribution of echoes was about equal over this range, the mean distance from bottom was taken as 5 m. However, if echoes were concentrated between 5 and 10 m from bottom, a mean of 7.5 m would be taken. In areas of heavy concentrations of fish, where echo traces were continuously recorded, an estimate of distance from bottom was made about once per hour. When echoes were recorded in distinct groups several miles apart, an estimate was made whenever possible. With the onset of darkness the vertical ascent is sudden. Certainly the distance moved off bottom in the deeper water is greater than in the shoaler water. To a depth of 128 m the mean nocturnal ascent varied between 5 and 20 m. In deeper water the vertical migration ranged from 5 to 51 m.

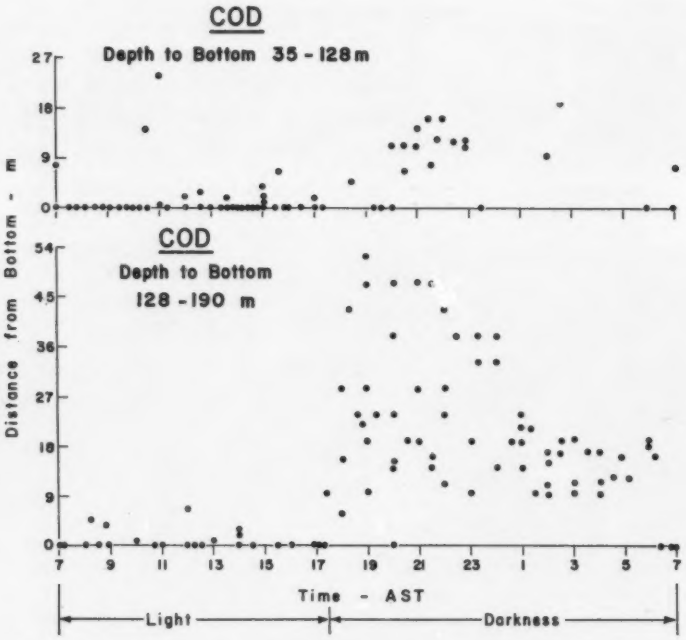

FIG. 4. Day and night vertical distribution of cod. Each point represents the mean distance from bottom for a group of fish. The data summarize observations made between 1960 and 1963.

Variations in the vertical migrational pattern did occur. On a cruise east of Cape Breton in about 182 m during January 1964, echo traces were observed off the seabed throughout the 24-hr period. However, they were farther off bottom after dark than during daylight. Between 0700 and 1000 AST the mean distance of traces from bottom was 18 m. Later in the day the cod moved closer to bottom so that between 1300 and 1600 the distance was 5 m. After dark the cod again moved farther off bottom, reaching a maximum of 22 m between 0100 and 0400. All cod examined were either immature or ripe.

HADDOCK, *MELANOGRAMMUS AEGLEFINUS* (LINNAEUS)

Echo traces of haddock were frequently observed off bottom at night and on bottom by day. Customarily, haddock did not move far from bottom. Concentrations on bottom by day and less than 5 m off bottom at night are illustrated in Fig. 5. The recordings were made near Sable Island at a depth of 80 m on May 2, 1961. A summary of observations on vertical migrations made on cruises between 1960 and 1963 is presented in Fig. 6. Each point in the figure represents a mean distance from bottom for a group of echoes. Echo traces were rarely observed off bottom during hours of daylight and usually not more than 9 m at night. Nevertheless, some traces were registered

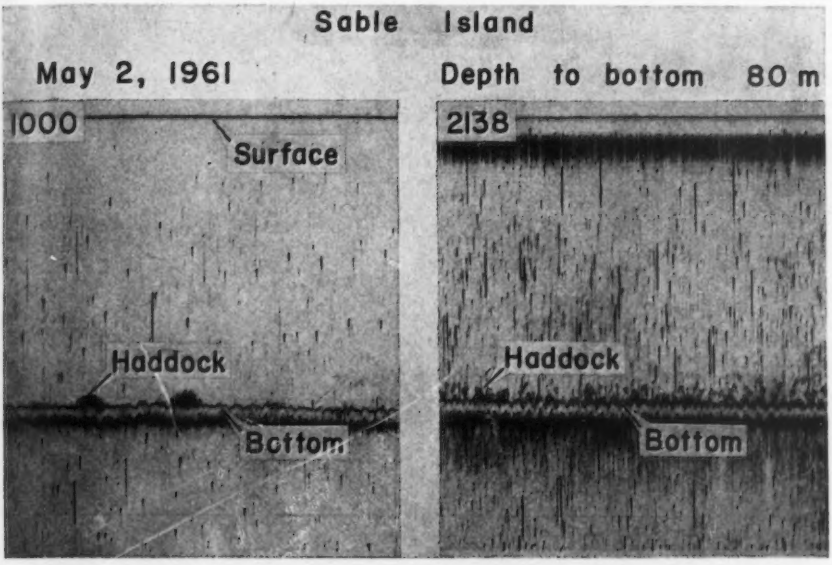

FIG. 5. Echo traces of haddock in contact with bottom by day (1000 AST) and less than 5 m off bottom at night (2138 AST).

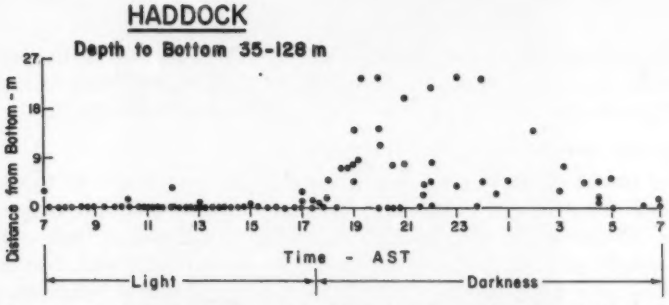

FIG. 6. Vertical distribution of haddock by day and night. Each point represents the mean distance from bottom for a group of fish. The data summarize observations made between 1960 and 1963.

considerable distances from the seabed. An echogram with individual traces, attributed to haddock, up to 60 m from bottom is given in Fig. 7. The recording was made in the Gully south of Banquereau on March 1, 1963, at a depth of 170 m. Dispersion of the concentration off bottom is evident from the figure. Usually, however, with the short distances moved from bottom, haddock tend to remain more concentrated than cod.

In contrast to the observation that haddock seldom move off bottom by day, captains of commercial vessels insist that this pattern does not always

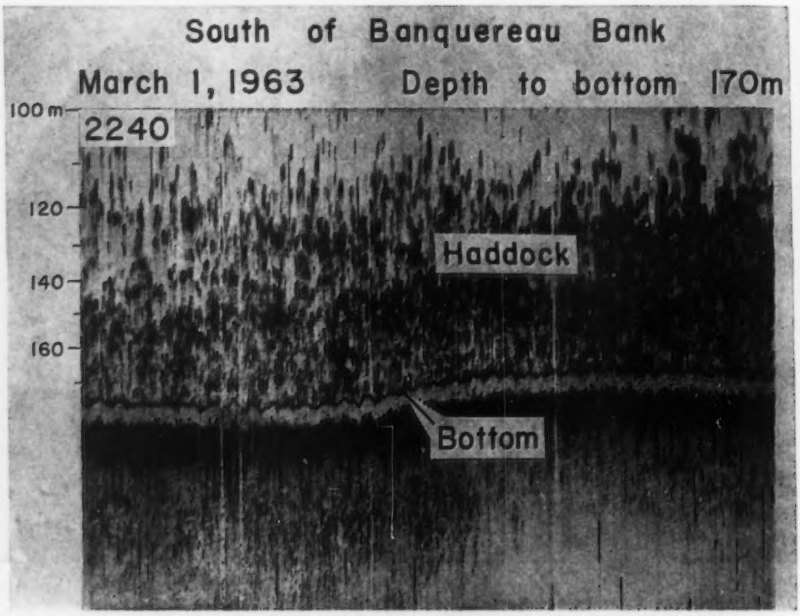

FIG. 7. Echo traces of haddock well off bottom at night. Traces extend from bottom upwards to about 69 m.

occur. There is nearly total agreement among fishermen that in the spring and occasionally at other times on northeastern and western Sable Island Bank, haddock remain on bottom by night and move to mid water by day.

REDFISH, *SEBASTES MARINUS* (LINNAEUS)

Echo traces of redfish were not observed as frequently as those of cod or haddock. This is not a reflection of relative abundance but rather one of emphasis. Cruises were not designed to study redfish, hence their occurrence in echo soundings and catches is more accidental than planned. Nevertheless, many echograms were obtained on the vertical migrational behaviour of redfish. Considerable variation in behaviour occurred by day. Mostly they formed in clusters on bottom. Often clusters extended as far as 15 m from bottom. One example is given in Fig. 8A. In other instances redfish carpeted the seabed in an area, much the same as cod and haddock (Fig. 8B). On dull or overcast days traces of redfish have been observed off bottom in compact small shoals (Fig. 8C). After dark, they invariably were observed off bottom. Most often they dispersed off bottom, although instances of compact shoals have been recorded.

FLATFISH

Of the different flatfish, American plaice, *Hippoglossoides platessoides* (Fabricius), was the only species observed off bottom. Plaice were recorded

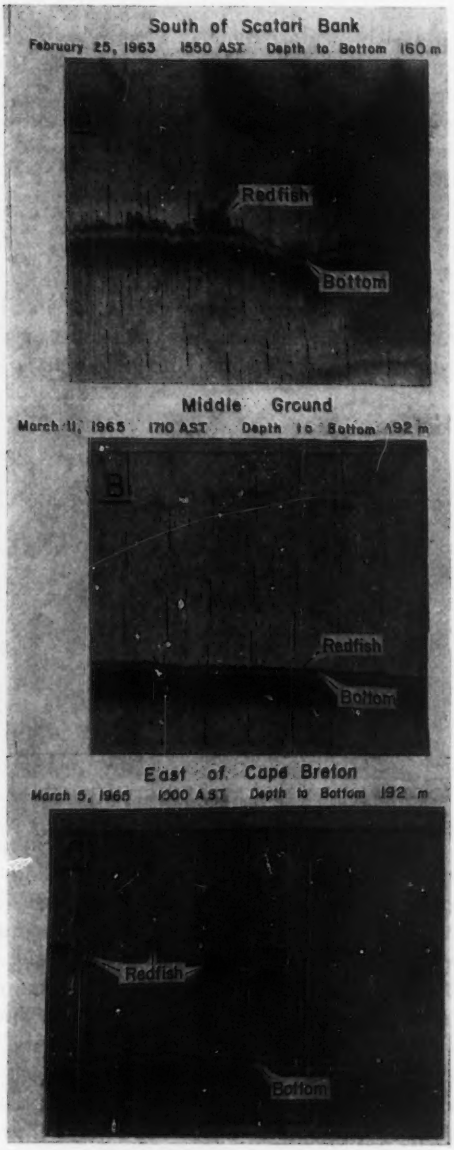

FIG. 8. **A.** Echo traces of redfish, in the form of clusters. Fish extend 15 m upwards from bottom. **B.** Redfish evenly distributed on the seabed by day. This is indicated by constant thickening of bottom line. **C.** Compact small shoals of redfish off bottom on an overcast day. Shoals are approximately 45 m off bottom.

off bottom at night east of Scatari Bank in 174 m during late February 1963. By day traces remained in close contact with bottom (Fig. 9). After dark traces were observed in large numbers off bottom (Fig. 9). The vertical ascent was up to 30 m, with most of the echoes between 11 and 30 m from bottom. Examination of plaice captured on bottom by day showed them to be mainly large fish in spawning or spent condition.

Day and Night Variations in Catch Size

cod

That cod move off bottom by night is reflected in otter-trawl catches. Generally, day catches are considerably larger. However, as might be expected, the variation in numbers caught by day and by night is large. Even in re-

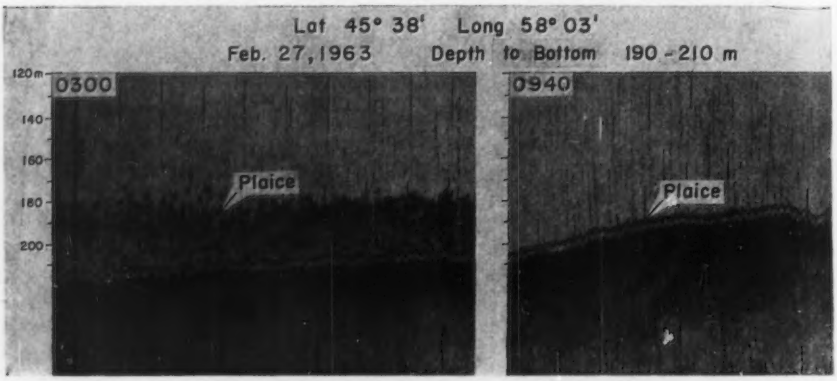

Fig. 9. Echogram of American plaice off bottom at night (0300 AST) and on bottom by day (0940). The observation was made east of Scatari Bank on February 27, 1963.

search cruises specifically designed to determine day and night differences, large variations occur. Consequently it is not possible to demonstrate statistically significant differences between means. This does not imply day and night differences are biologically insignificant.

Of the areas fished by the *A. T. Cameron*, largest numbers of cod were caught in the Gulf of St. Lawrence and Cape Breton area. The results, separated by depth and season, are summarized in Table I. Day and night fishing was conducted over 4 months, January, March, April, and October. Generally, catches were larger by day. There was some indication of seasonal variation in the relation between day and night catch size. This is illustrated in Fig. 10, where mean night catches, expressed as per cent of day catches, are plotted against season (month) for each depth range. Within each depth range, diurnal differences were most pronounced in March and least in January and October. The depth fished had little noticeable influence on the relation between day and night catches.

TABLE I. Diurnal changes in mean numbers of cod taken in the Gulf of St. Lawrence and Cape Breton area by the CGS *A. T. Cameron*. The number of tows is given in parentheses beside the mean number of cod per tow. Two cruises each were made in January and April, one each in March and October.

Depth range (*m*)	January		March		April		October	
	Day	Night	Day	Night	Day	Night	Day	Night
0–53							427 (17)	342 (6)
54–108	833 (7)	887 (3)	602 (1)	95 (1)	160 (38)	47 (32)	203 (10)	191 (10)
109–163	1215 (9)	661 (8)	1240 (4)	384 (4)	356 (13)	184 (14)	324 (11)	192 (4)
165+	684 (8)	448 (10)	636 (1)	61 (3)	168 (7)	91 (3)	45 (6)	36 (5)

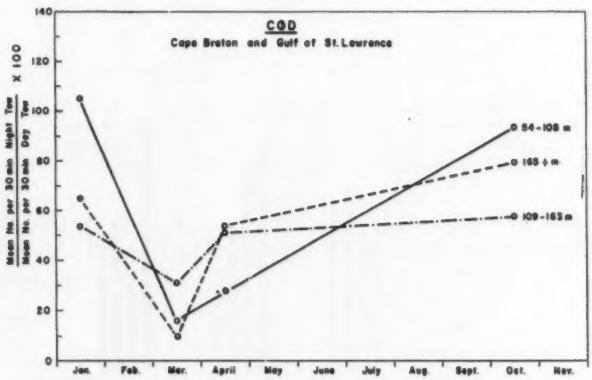

FIG. 10. Seasonal differences in the relation between day and night catches of cod. The values were calculated from the data in Table I.

Comparison between day and night catches of cod was made by the *Harengus* on a cruise off Miscou Island in the Gulf of St. Lawrence during September 1962. Fishing was restricted to a small area of nearly constant depth. Day tows were made between 1200 and 1700 ADT; night tows began after 1900 and continued to about 2300 ADT. Sunrise and sunset occurred at approximately 0630 and 1819, respectively. Individual daylight catches were consistently larger than those made after dark. The results are presented in Fig. 11. The mean number of cod taken by day (680) was almost double that by night (360).

Fishermen almost unanimously report the larger catches of cod by day. Typical variations in catch over 24 hr are presented in Fig. 12. In the figure, each point represents the mean catch for all tows made within that hour. The mean time for each tow was taken as the time of the haul. The catch for every tow was equated to 30 min. For example, if the duration of tow was 2 hr and

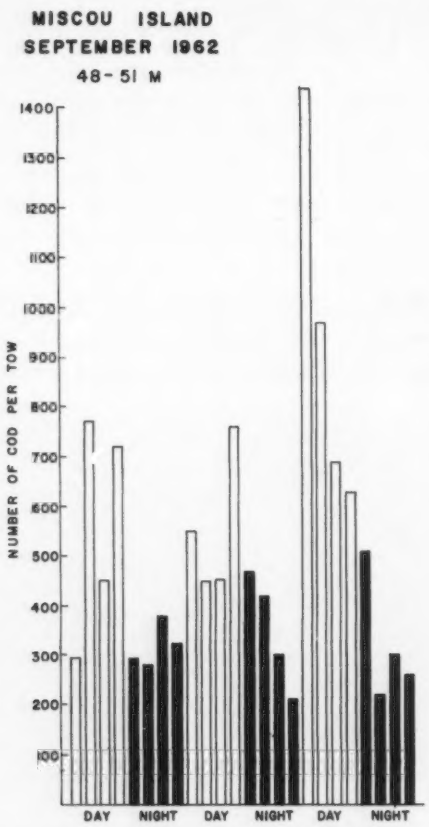

FIG. 11. Diurnal variation in number of cod caught per tow by the MV *Harengus* over a 3-day period. Daylight tows began at about 1200 ADT and continued to about 1700; night tows began after 1900 ADT and continued for approximately 5 hr.

the catch 2000 lb, the catch for 30 min was estimated as 500 lb. The three cruises were made during June and July, two on Sable Island and one on Georges Bank. Large catches of cod were made on Sable Island in June 1963, with the day catches about twice those after dark. Smaller catches were made on Sable Island in July 1964 with day catches more than twice as large as night hauls. The June cruise to Georges Bank followed a pattern similar to the 1964 Sable Island cruise, with more than twice the poundage of cod per tow by day than by night. Except for the western portion of Sable Island, fishermen report larger catches by day on the Nova Scotian Banks over all seasons. On western Sable, especially in spring, night hauls are reportedly larger.

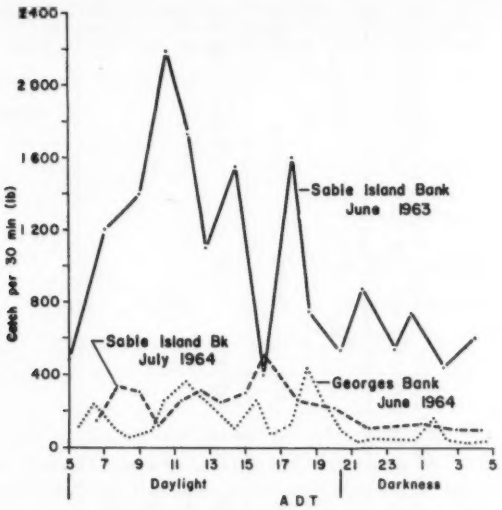

FIG. 12. Diurnal variations in catch of cod by three commercial otter trawlers. Each point represents the mean poundage for all tows made within the hour. In several instances, no tows were made within an hour period.

Fishermen attribute this to cod leaving the seabed by day and returning to bottom by night. Echograms and catch statistics to illustrate this behaviour were not available.

HADDOCK

Generally, otter-trawl catches complement echo-sounder observations. Ordinarily day catches are the larger, although differences are less than those found for cod. Comparisons between mean numbers caught by day and night by the *A. T. Cameron* on Sable Island and Emerald Banks from 1958 to 1964 are summarized in Table II. Fishing was carried out over 5 months covering winter, spring, summer, and fall. Six cruises were made in winter (two in February, four in March), one in spring (May), one in summer (July), and one in fall (October). Most tows were made between 54 and 164 m.

In most cases daytime catches were from just over one to six times greater than those at night. In only two instances were the numbers by day clearly less than those by night. In March between 54 and 108 m and in October to a depth of 53 m, day catches were smaller. Numbers of haddock per tow were also smaller by day in May in shoal water; however, few fish were captured in the four tows made.

Comparison of day and night catches in the Gulf of St. Lawrence and off Cape Breton is made in Table III. Two cruises each were made in January and April, one each in March and October. Larger numbers per tow were taken by day, although catches were comparatively small. Numbers per tow ranged

TABLE II. Diurnal changes in the numbers of haddock taken on Sable–Emerald Banks by the CGS *A. T. Cameron*. The number of tows is given in parentheses beside the mean number of haddock per tow. Results from cruises made between 1958 and 1964 have been combined.

Depth range (*m*)	February		March		May		July		October	
	Day	Night	Day	Night	Day	Night	Day	Night	Day	Night
0–53					16 (2)	29 (2)	195 (13)	34 (8)	125 (8)	242 (4)
54–108	661 (29)	628 (21)	241 (17)	304 (18)	436 (36)	358 (22)			256 (19)	174 (11)
109–164	161 (10)	132 (15)	170 (16)	90 (39)	246 (8)	93 (5)				
165+			22 (9)	13 (4)	25 (3)	9 (3)				

TABLE III. Diurnal changes in the numbers of haddock taken in the Cape Breton area by the CGS *A. T. Cameron*. The number of tows is given in parentheses beside the mean number of haddock per tow. Results have been combined from cruises made between 1959 and 1964.

Depth range (*m*)	January		March		April		October	
	Day	Night	Day	Night	Day	Night	Day	Night
0–53							6 (18)	1 (6)
54–108					440 (38)	284 (26)		
109–164	6 (9)	1 (8)	27 (4)	18 (4)	63 (13)	48 (14)	4 (11)	2 (4)
165+	63 (9)	32 (10)	12 (1)	14 (3)	14 (7)	14 (3)	92 (6)	70 (5)

from just over one to two times larger by day. Only in March and April at a depth of more than 165 m was the number by night as large as that by day. In both instances the number per tow was not large nor were many tows made.

For both areas (Cape Breton–Gulf of St. Lawrence and Sable–Emerald) the relation between day and night catch size varied with the month fished (Fig. 13). Certainly, there is strong suggestion that, for the months examined diurnal differences are the least distinct between February and April, with the largest differences occurring in January and October. Interestingly, this pattern is the inverse of that found for cod.

Seven cruises by commercial vessels were examined for day and night variations in catch. Haddock was the main species caught on all cruises. The

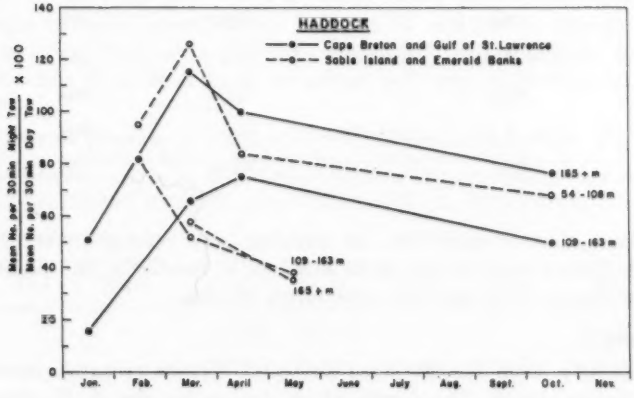

FIG. 13. Seasonal differences in the relation between day and night catches of haddock. The values were calculated from the data in Tables II and III.

results of each cruise are presented in Table IV with the number of tows made by day and by night, time and area fished. Tows made within the hours of sunrise and sunset were omitted. All catches were converted to poundage per 30-min tow by the method cited for cod.

Mean poundage per tow was lower by day for each of the three 1959 cruises and the one 1960 cruise. Day catches ranged from half to three-quarters the poundage of night tows. Of the three cruises in 1964, two made larger catches by day. Catch per tow by the MV *Drift* during daylight hours was about

TABLE IV. Summary of seven cruises made by commercial fishing vessels on Nova Scotian Banks between 1959 and 1964. All tows made during the hours of sunrise and sunset have been omitted.

Vessel	Date	Area	Time	Mean poundage per 30-min tow	No. of tows
Cape Charles	June 9–17, 1959	Sable Island Banquereau	Day	315	33
		St. Pierre	Night	636	19
Cape Brier	June 30–July 8, 1959	Sable Island Banquereau	Day	328	37
			Night	425	23
Cape Charles	July 21–27, 1959	Sable Island	Day	420	30
			Night	589	13
Cape Charles	July 8–19, 1960	Sable Island	Day	218	29
			Night	438	16
Drift	June 12–15, 1964	Browns	Day	359	8
			Night	491	5
Cape Sable	June 17–25, 1964	Georges	Day	663	53
			Night	365	23
Acadia Crest	Aug. 14–24, 1964	Sable Island	Day	908	16
			Night	758	10

three-quarters that of nighttime. In contrast, day catches by the MV *Cape Sable* were almost twice those made after dark. Similarly, the poundage per tow by the *Acadia Crest* was 1.2 times larger by day.

REDFISH

Consistently large catches were not made by the research vessels (Jean and McCracken, 1961). The results of six cruises by the *A. T. Cameron* between 1959 and 1963 are presented in Table V by depth. The areas fished included the Gulf of St. Lawrence and the deeper waters around the edges of

TABLE V. Diurnal differences in catches of redfish. The number of tows is given in parentheses beside the mean number of redfish per tow. The results of six cruises between 1959 and 1963 have been combined. The areas fished included Sable Island Bank, Banquereau, and the Gulf of St. Lawrence area.

Depth range (*m*)	Day	Night
109–164	253 (108)	89 (83)
165+	575 (40)	208 (25)

Sable Island and Banquereau Banks. Clearly the greater numbers per tow were captured by day. In both depth ranges (109–164 and more than 165 m) numbers caught by day were about 2.8 times those taken after dark.

Records from commercial boats are not available. However, questionnaires and interviews show fishermen are in complete agreement that day catches are larger. Indeed, they sometimes report that it is not profitable to fish for this species after dark. Several skippers have observed smaller catches on dull rather than bright days.

FLATFISH

Four species are caught in commercial numbers: plaice; winter flounder, *Pseudopleuronectes americanus* (Walbaum); greysole, *Glyptocephalus cynoglossus* Linnaeus; yellowtail, *Limanda ferruginea* (Storer). Day and night differences in numbers per tow were determined from data collected by the *A. T. Cameron* and *Harengus*. These are summarized in Table VI.

Numbers of plaice per tow were larger by day except in less than 53 m. The results in the Table are based on four cruises, three to the Cape Breton area and one to Sable Island Bank. Between 54 and 164 m daylight catches were about twice those at nighttime. Below 165 m day catches were more than three times those after dark. However, only two night tows were made.

TABLE VI. Diurnal differences in numbers of flatfish captured by otter trawl. The number of tows is given in parentheses beside the mean number of fish per tow. All data for witch, yellowtail, and plaice were obtained by the *A. T. Cameron*, those for winter flounder by the *Harengus*.

Depth range (*m*)	Greysole		Yellowtail		Witch flounder		Plaice	
	Day	Night	Day	Night	Day	Night	Day	Night
0–53			37 (28)	75 (29)			42 (30)	65 (14)
54–108			19 (64)	64 (30)	170 (8)	231 (7)	188 (79)	99 (57)
109–164							187 (31)	98 (29)
165+	73 (9)	190 (15)					143 (13)	45 (2)

Day and night catches of winter flounder were made by the *Harengus* in Passamaquoddy Bay region of the Bay of Fundy during late November 1962. Slightly smaller numbers were taken by day than by night. The mean number per tow by day (170) was approximately three-quarters of that by night (231). The depth fished ranged from 46 to 64 m.

Relatively little data are available for greysole. One cruise by the *A. T. Cameron* in February 1964 was designed as a greysole survey trip. Tows were made in two small areas, one near Scatari Bank, the other, "Greysole Hole," at depths of 273–291 m. As indicated in Table VI, the number per tow by day (73) was less than half that by night (190).

Day and night differences in numbers of yellowtail per tow were based on three cruises by the *A. T. Cameron* to Sable Island and Emerald Banks. Numbers in day hauls were less than half those in night tows. To a depth of 53 m the mean number per tow was 37 by day for 28 tows and 75 by night for 29 tows. Between 55 and 108 m, differences were larger with a mean of 19 per tow by day for 64 tows and 64 by night for 30 tows. In the deeper water day catches were less than one-third of those made after dark.

Day and Night Variations in Size Composition

The usual method of demonstrating day and night differences in size composition is to compare mean numbers within a particular length range. Although such a procedure may show differences, and is used in this study, more refined statistical treatment will demonstrate whether or not differences are significantly greater than variation within day or night catches. Two methods have been followed. Within a particular size range the mean number per tow was calculated along with the standard error times t, where t is the value for $n-1$ at the 95% level (Snedecor, 1946). If the mean number per tow plus or minus t times the standard error by day does not overlap that by night, differences were considered significant. Secondly, comparisons were made between deviations in length composition of individual tows and the general mean by a modification of the χ^2 test. The method, described by Kupperman (1959), easily permits calculation of χ^2 values within and between samples. Hence comparisons were made within and between day and night tows. In each case, estimates of χ^2 and χ^2/df indicated a less than 1% probability that samples arose from a homogeneous population. If the heterogeneity estimate between day and night is greater than that within, the suggestion is that length composition by day and night is not similar. On the other hand, if the between heterogeneity estimate is equal to or less than those within, length composition by day and by night is not regarded as significantly different.

COD

Five cruises were selected for their large numbers of cod. Tows were first separated by depth and then day or night. Catches were broken down to several length classes, in this case, seven. The mean number in each length class is presented in Table VII for each cruise.

TABLE VII. Size composition of cod caught by the CGS *A. T. Cameron* on five cruises.

Cruise	Area	Depth (*m*)	No. of tows	Time	Average no./tow by size (*cm*)							Total	χ^2/df within	χ^2/df between
					<19	25	34	43	52	61	67>			
80	Gulf of	165+	4	Day	0	40	246	430	304	137	116	1278	36.8	36.2
	St. Lawrence		5	Night	0	11	80	160	147	60	28	486	11.5	
		109–164	6	Day	3	50	187	601	429	64	22	1356	49.1	29.2
			6	Night	12	57	78	372	260	48	18	845	101.2	
54	Northern	109–164	9	Day	5	83	138	182	66	23	12	509	24.6	127.3
	Gulf of St.		6	Night	3	111	121	85	46	27	11	404	10.4	
	Lawrence	54–108	6	Day	7	57	52	187	289	96	10	698	58.0	34.3
			7	Night	2	19	18	18	10	4	3	72	6.4	
37	Sable–	54–108	20	Day	6	7	4	12	43	63	73	208	17.9	17.5
	Emerald		7	Night	2	5	3	3	6	8	19	46	4.8	
32	Gulf of	165+	4	Day	7	13	29	24	11	6	5	99	2.8	15.9
	St. Lawrence		5	Night	58	52	65	65	49	43	75	407	26.6	
29	"	109–164	11	Day	8	12	87	106	68	29	14	324	5.0	1.8
			4	Night	7	8	50	62	41	13	10	195	2.8	
		54–108	10	Day	8	24	63	51	24	12	7	189	11.5	7.7
			10	Night	11	19	70	55	21	9	6	191	11.1	
		0–53	17	Day	7	51	226	102	25	8	5	404	14.9	69.9
			6	Night	37	62	146	58	25	10	4	342	16.5	

In all but one case mean numbers of large fish longer than 39 cm were the greater by day. Small fish were less often taken in the larger numbers by day. In the one exception (cruise 32) night catches were consistently the larger over all lengths.

All data within a depth range were combined and means examined for statistically significant diurnal differences (Table VIII). Obviously, the results in the Table offer no suggestion of diurnal differences for either small or large cod. Heterogeneity in size composition within day and within night was compared with that between. The results are given in Table VII. In only two of nine cases was heterogeneity between larger than that within day and night. Hence, there is little suggestion of statistically significant differences in size composition.

TABLE VIII. Day and night comparisons of mean numbers of large and small cod caught by otter trawl. The standard error times t is presented for each mean.

Depth (*m*)	No. of tows	Time	Mean $\pm t$ · standard error		
			Small	Large	Total
54–108	36	Day	55±26	230±184	285±187
	24	Night	57±27	59±18	116±35
109–164	26	Day	179±126	449±354	628±450
	16	Night	160±80	358±295	518±312
165+	8	Day	168±268	516±603	684±846
	10	Night	134±97	315±151	449±203

HADDOCK

Evidence for day and night differences in size composition is based on results collected by the *A. T. Cameron*. Six cruises were selected because of large numbers caught. Five were to Sable and Emerald Banks and one to the Gulf of St. Lawrence. Tows were separated into several depth ranges and within each, by day and night. Fish were classified into nine length groups. Mean numbers per tow by day and night are given for each cruise in Table IX. Most often, the mean number of small fish (less than 38.5 cm) was the larger by day. The relation is less clear for large fish. Often night catches were greater than or at least equal to day hauls. The results of the six cruises were combined and the mean number of small and large haddock per tow, between 54 and 108 m, was calculated by day and night. Small haddock were considered as less than 38.5 cm. The standard error times t was calculated for each mean. By day the mean number of small fish per tow was 247 ± 24 and by night, 86 ± 85. Here the number by day is significantly greater than that by night. The mean

TABLE IX. Size composition of haddock caught by the CGS *A. T. Cameron* on six cruises.

Cruise	Area	Depth (*m*)	No. of tows	Time	Average no./tow by size (*cm*)									Total	χ^2/df within	χ^2/df between
					<20.5	24.5	28.5	32.5	36.5	40.5	44.5	48.5	50.5>			
5	Sable–	54–108	6	Day	57	50	33	43	32	19	16	10	33	293	33.7	11.3
	Emerald		5	Night	25	49	27	36	31	16	21	15	40	260	18.1	
		109–164	4	Day	91	17	16	20	16	18	14	13	13	218	44.1	52.5
			7	Night	9	22	15	18	25	25	9	9	21	153	12.1	
18	Emerald–	54–108	22	Day	20	15	82	233	152	134	63	43	80	822	52.9	623.5
	Sable		16	Night	4	5	28	44	81	194	143	99	145	743	34.5	
30	Gulf of	0–53	8	Day	3	41	73	27	82	103	44	21	12	406	7.9	360.6
	St. Lawrence		4	Night	1	9	27	16	124	153	69	17	17	423	42.9	
		54–108	17	Day	6	27	36	21	53	59	36	25	21	284	12.1	35.0
			9	Night	6	13	12	6	56	68	32	9	7	209	15.6	
37	Sable–	54–108	30	Day	17	27	21	11	38	93	94	66	73	440	39.4	237.9
	Emerald		21	Night	2	4	5	2	11	60	111	79	79	353	9.7	
53	Sable–	54–108	24	Day	38	7	22	109	102	56	92	68	76	570	38.0	156.3
	Emerald		21	Night	13	2	6	32	31	33	73	60	78	328	19.1	
66	Sable–	54–108	5	Day	73	25	32	12	36	75	60	45	82	440	33.4	229.7
	Emerald		7	Night	25	3	6	1	2	17	28	64	318	464	36.4	

number of large fish per tow by day, 271 ± 54, was less than, but not significantly, that by night, 331 ± 132.

Examination of the χ^2/df approximates in Table IX indicates that with one exception heterogeneity within day and within night is less than that between. Hence the suggestion is that size composition by day is different from that by night.

Only in cruise 5 (55–108 m) does the analysis not support the suggestion of differences in size composition. Here heterogeneity within day and within night is greater than that between.

FLATFISH

Comparatively large numbers of yellowtail were taken on three cruises by the *A. T. Cameron*, two to Sable Island Bank and one to the Gulf of St. Lawrence. All of the catches were taken in less than 53 m. Fish were separated by day and by night into six length groups. The mean numbers per tow are given in Table X along with areas fished and number of tows made. Greater numbers over all length classes were usually taken by night than by day. However, the ratio of night to day catches was higher for small fish (less than 22.5 cm). To better illustrate differences, results from the three cruises were combined. Mean numbers of small and large yellowtail were calculated along with the standard errors times *t*. In night tows, the mean number of small fish (75 ± 42) was significantly greater than by day (13 ± 4). In contrast, no significant difference was observed for large fish. The mean number taken by day was 88 ± 48, and by night, 146 ± 67.

Estimates of heterogeneity within day and within night tows as well as between are given in Table X. In two of three cruises, heterogeneity within both day and night is less than that between. In the other, heterogeneity within night is greater than that between day and night. Hence, in two of three cases, there is statistical support for day and night differences in size composition.

Evidence of day and night differences in size composition of American plaice is based on data collected on three cruises by the *A. T. Cameron*. For each cruise, tows were separated according to depth and by day and night. Fish were classified into six length groups. The mean number per tow in each length class is given in Table XI.

On cruise 18, day catches were lower over all length classes. In another cruise (54) catches of small fish were the greater by day between 110 and 163 m, and catches of large plaice the larger by night. At other depths, catches by day were the larger for all length classes. Similarly on cruise 29, more plaice of all sizes were taken by day than by night.

A comparison of mean numbers per tow by day and night was made for large and small plaice from the combined data of the three cruises over the range 54–108 m. A similar comparison was made between 110 and 163 m. In neither instance was the mean number of small or large fish by day significantly different from that by night. Over the range 54–108 m the mean number

TABLE X. Size composition of yellowtail caught by CGS *A. T. Cameron* on three cruises.

Cruise	Area	Depth (*m*)	No. tows	Time	Average no./tow by size (*cm*)						Total	χ^2/df within	χ^2/df between
					<16	19.5	25.5	31.5	37.5	41>			
29	Gulf of	0–53	9	Day	1	11	15	13	6	0	46	3.3	25.3
	St. Lawrence		4	Night	15	61	33	21	6	0	136	2.1	
30	Sable Island	0–53	7	Day	2	9	10	9	14	5	49	1.3	10.9
	Bank		5	Night	13	48	18	35	60	7	181	43.7	
88	Sable Island	0–29	6	Day	1	18	72	117	31	5	244	7.5	329.0
	Bank		4	Night	19	74	123	113	26	2	457	25.5	

TABLE XI. Size composition of plaice caught by the CGS *A. T. Cameron* on three cruises.

Cruise	Area	Depth (*m*)	No. tows	Time	Average no./tow by size (*cm*)							Total	χ^2/df within	χ^2/df between
					<16	16–19.5	19.5–25.5	25.5–31.5	31.5–37.5	37.5–41	41>			
18	Sable–Emerald	109–164	4	Day	0	10	18	9	3	1		41	2.1	0.9
			7	Night	2	15	21	15	6	2		68	3.2	
29	Gulf of St. Lawrence	0–53	9	Day	44	79	46	52	7	9		246	6.3	11.5
			5	Night	9	27	35	18	5	3		97	2.0	
		54–108	9	Day	71	107	196	120	24	11		529	18.3	14.5
			10	Night	50	67	152	69	8	2		348	9.9	
		165+	12	Day	31	35	54	32	9	8		169	2.0	9.9
			4	Night	4	7	19	15	2	1		52	2.4	
54	Northern Gulf of St. Lawrence	54–108	7	Day	13	42	84	100	42	19		300	11.9	5.9
			6	Night	3	15	48	65	31	17		179	6.9	
		109–164	9	Day	14	18	33	27	11	33		136	24.6	95.0
			5	Night	2	8	57	96	82	118		363	10.1	

of small plaice was 124 ± 64 by day and 80 ± 43 by night. For large fish the mean was 304 ± 153 by day and 206 ± 22 by night. Between 109 and 164 m the mean number of small fish was 25 ± 19 by day and 21 ± 22 by night. In the case of large plaice, the mean number by day, 81 ± 61, was less than but not significantly different from that by night, 193 ± 132.

Heterogeneity estimates within and between day and night tows are presented in Table XI. In three of six cases heterogeneity between was greater than that within day or night. Further, in two of these three cases, differences were not large.

DISCUSSION AND CONCLUSION

As a general rule, nocturnal vertical migrations were observed for cod, haddock, and redfish. After dark, echo traces were often recorded considerable distances off bottom. Traces of cod have been observed more than 73 m from bottom. Similar observations were reported by Cushing (in Richardson et al., 1959). He also observed cod 73 m off bottom after dark. Saetersdal (1956), Trout (1957), Konstantinov (1958), Parrish et al. (1964), and Woodhead (1964) all report migration of cod to mid water at night. Exceptions to this pattern have been observed. On one cruise in January to the Cape Breton area, cod were found off bottom by day and night. However, by day, traces were closer to bottom than after dark. On the Vestfjord spawning grounds off the Lofoten Islands in northern Norway, Sundnes (1964) and Woodhead (1964) observed echo traces of cod off bottom by day and night. Further, cod remained in compact shoals off bottom. Other instances of cod off bottom by day and night have been reported by Brunel (1964), Templeman (1964), Trout (1957), and Ellis (1956).

That haddock are more closely associated with the seabed in their vertical migrations than other gadoids is consistent with the view of Woodhead (1964). Moreover, that they occasionally move far from bottom is concordant with the observations of Hjort (1914) and Trout (1962). Hjort captured some haddock near the surface in depths of over 1000 m. Trout (1962) caught haddock in a midwater trawl some 123 m from bottom.

Generally redfish move off bottom, often considerable distances, with the onset of darkness and return to the seabed by day. Again exceptions were recorded. On dull days, redfish sometimes remained off bottom in compact shoals.

Certainly changes in light intensity appear to elicit the migrational response. Similar views have been expressed by many investigators (Konstantinov and Scherbino, 1958; Parrish et al., 1964; and others). Little is understood of the ecological significance of vertical migrations. A popular suggestion is that fish feed in mid water. Certainly, some evidence of this is available for redfish. From food studies, Templeman (1959) concluded redfish are pelagic feeders. Echo traces attributed to redfish have been observed at night among concentrations of Ctenophora and Copepoda. None of the redfish were captured. However, Steele (1957) found that these plankton comprise a con-

siderable portion of the redfish diet. Food studies on cod are not in contrast to the suggestion that they feed off bottom at night. Parrish et al. (1964) observed cod feeding on herring at night near the surface in the Moray Firth. Indeed, Zatsepin and Petrova (1939) attribute vertical migrations by cod in the south part of the Barents Sea to the pursuit of food. Food has also been associated with changes in vertical migrational behaviour. Brunel (1964) observed cod off bottom by day from May to mid July in the Bay of Chaleur. He suggests food is responsible for the change in behaviour. Reports of cod living pelagically and feeding on capelin from mid June to early August in shoal water have been made by Templeman (1964) for the Newfoundland area. After spawning in deep water, cod follow capelin, which are on a spawning "run," to coastal waters. No evidence of haddock feeding off bottom was obtained. Examination of stomachs consistently indicates the diet to be of benthic fauna. Other less well-documented suggestions on the adaptive significance of vertical migrations are spawning (Brawn, 1961) and egg dispersal, and annual horizontal migrations (Trout, 1957).

In accord with echo-sounder observations, catches of cod, redfish and, to a lesser extent, haddock, were usually larger by day. Both cod and haddock exhibited seasonal fluctuations in the relation between day and night catch size. For cod, diurnal differences were most pronounced in March and less pronounced in January and October. In contrast, day and night catches of haddock were of the same order in March and April. Diurnal differences in catch size were greatest in January and October. Tentatively, seasonal variation is attributed to differences in the proportion of stocks leaving the seabed at night. Few echo traces were ever observed off bottom by day. Information for the western North Atlantic is particularly scarce in the literature. Brunel (1964) has made a careful study of cod in the Bay of Chaleur in the Gulf of St. Lawrence. He found daylight catches smaller from mid May to July and larger from July to October. In his research on redfish in the western North Atlantic, principally on the eastern Grand Banks, Templeman (1959) found larger catches by day for all depths over all seasons. Similar observations have been reported for redfish by Steele (1957), Macdonald (1927), Konstantinov and Scherbino (1958), and von Seydlitz (1962).

Investigations in the eastern Atlantic Ocean have, for the most part, found the larger catches of cod by day. Konstantinov (1958) found larger catches by day in the Barents Sea. Similarly, Parrish et al. (1964) noted that catches were larger by day during November and January in the Moray Firth. Woodhead (1964) reported greater catches by day than by night for each of eight winter cruises to Malangen and North West Banks off the northern coast of Norway. The mean day catch by weight for all cruises combined was slightly more than double that for night. Bagenal (1958) noted that the cod catches by day were two to three times as large as those made at night between April and October in the Clyde area. In contrast to these findings, Jones (1956) reported much larger catches by night (four to five times) than by day. The results were obtained in April off Faeroe Island. Reports on

diurnal differences in catches of haddock are less consistent than those for cod. Jones (1956) found day catches one-fifth to one-quarter as large as those made after dark in April in the Faeroe area. Smaller day catches of haddock were also observed by Woodhead (1961). In a November cruise to the North Deeps in the Barents Sea, daylight catches were about half those made after darkness. In contrast, Bagenal (1958) found day catches ranged from 5 to 33 times those made at night between April and October in the Clyde area. Similarly, Woodhead (1961) found daylight hauls some four times the larger on Skolpen Bank in the North Sea during a November cruise. Parrish et al. (1964) found considerable variation in the differences between day and night catches. Of eight cruises in the Moray Firth, five yielded smaller catches by day.

Evidence was obtained to suggest day and night differences in size composition of haddock. Fewer small haddock (less than 38.5 cm) were taken by night than day. Catches of large fish tended to be about the same or slightly larger by night. This is attributed to small haddock leaving the seabed at night in greater proportions than large fish. Some reference to day and night variation in size composition of haddock in otter-trawl hauls is available in the literature. Woodhead (1964) found contrasting differences in size composition of haddock by day and night in the eastern North Atlantic. In one area he caught greater numbers of small haddock by day than by night. In another area, greater numbers of small haddock were taken after dark. Parrish et al. (1964) could not detect differences in size composition over day and night tows in the Moray Firth. Finally, Jones (1956) reported larger numbers of small haddock by day than by night in the Faeroe area and a greater total catch by night.

Diurnal differences in size composition may explain the larger catches (poundage) by commercial vessels at night and the greater numbers per tow by day by research vessels. Commercial otter trawls are of large mesh throughout while in research trawls the codend is lined with small-mesh netting. Hence escapement by small fish would be greater in commercial than in research gear. For research gear small haddock are more numerous in daylight hauls and large fish tend to be more abundant in night tows. If only large haddock are considered, research-vessel results agree with those of commercial vessels.

Greater catches of large haddock by night may relate to diurnal variations in behaviour ahead of a trawl. Blaxter et al. (1964) have made observations in tanks on the reaction of haddock and other species to an approaching obstacle or net under visual and nonvisual conditions. By day, fish are herded better by towed gear, but actively swim ahead of it, thereby increasing chance of escapement. Under nonvisual conditions, herding breaks down but fish are more easily captured.

That significant diurnal differences in size composition were not observed for cod is in contrast to the findings of Parrish et al. (1964). They found more small cod in day than night tows, and little difference in the numbers of large fish. Diurnal changes in size composition were not examined for redfish.

However, Templeman (1959) did not find any clear indication of size difference by day and night.

Not all species taken in larger numbers by day move off bottom at night. Except in shoal water, plaice were caught in larger numbers during daylight hours, but do not regularly leave bottom at night, spawning fish being a notable exception. In his examination of commercial catches of plaice, as well as those made by a research vessel, Pitt (personal communication) also found larger catches were made by day on the Grand Bank than after dark. Bagenal (1958) reported larger day catches of the closely related long rough dab from the eastern Atlantic, while Parrish et al. (1964) found larger catches by night.

It is suggested that the greater daylight catches are related to increased feeding activity. Analyses of over 400 stomachs support the suggestion. By day, the ratio of freshly-eaten food to digested was twice that by night. Presumably plaice feed by day and remain inactive on bottom by night. Thus there would be a tendency for the towed otter trawl to pass over the inactive, perhaps partially buried, plaice.

That plaice in spawning condition were observed off bottom at night is in agreement with observations of others on North Sea soles (Verwey, 1960; Møller Christensen, 1962). DeVeen (1964) reported soles in prespawning condition swimming well off bottom on dark moonless nights.

Winter flounder, greysole, and yellowtail were taken in larger numbers by night. Similar findings for winter flounder were reported by McCracken (1964) in Passamaquoddy Bay during mid June 1949. However, in mid August, the mean number by day exceeded that by night. Bagenal (1958) found day catches of greysole up to three times larger than those made after dark in the Clyde area in the eastern North Atlantic. On the other hand, Parrish et al. (1964) observed larger catches of greysole at night.

One explanation is to attribute diurnal differences in catch size to changes in activity. This is concordant with the views of Kruuk (1963) and Woodhead (1963). Further, it is not improbable that larger night catches are related in some degree to changes in the reaction of fish to the trawl. Reduction in avoidance to gear at night (Blaxter et al., 1964) could increase catches, especially if flatfish are not in close contact with the seabed. Presumably reduced avoidance is related to visual conditions. Thus, small fish might escape through meshes in wings of trawls better under visual than nonvisual conditions. This might explain the larger numbers of small yellowtail taken by night than by day. Diurnal variation in size composition was not evident for American plaice. Presumably, at night, activity is at a minimum. Hence reduced avoidance under nonvisual conditions is not a significant factor.

In the eastern North Atlantic several investigators have reported on day and night differences in size composition of plaice, *Pleuronectes platessa*. Woodhead (1964) reported bigger plaice in daylight tows although differences were small enough to be of questionable significance. Parrish et al. (1964) observed larger numbers of small plaice, lemon sole, and dabs in night hauls. Boerema

(1964) could not determine any difference in size composition of plaice by day and night.

SUMMARY

Echo traces of cod and redfish were usually observed off bottom at night over all seasons and areas studied. By day, cod usually remain in close contact with bottom while redfish may form in clusters extending several metres above bottom. There was some evidence that on dull or cloudy days redfish may leave the seabed. Haddock followed the same pattern as cod but are more closely associated with the seabed. Nocturnal ascent by cod ranged mostly from 15 to 46 m, with occasional traces up to 73 m off bottom. Usually haddock remained within 9 m from bottom, although traces were recorded 70 m off bottom.

Concentrations of cod, haddock, and redfish recorded on bottom by day disperse in mid water at night. On occasion, compact shoals attributed to redfish have been recorded in mid water by day.

Echo traces of American plaice were recorded up to 20 m off bottom at night on one occasion (late February 1963). Examination showed them to be in prespawning condition. By day, they formed a dense layer on bottom; by night, they dispersed in mid water.

In most instances, daylight catches of cod, haddock, and redfish were larger. There was some indication of a seasonal variation in the relation between day and night catches for cod and haddock. Diurnal differences for cod were greatest during March and least during January and October; for haddock, largest differences occurred in January and October, smallest between February and April. Except in shoal water, plaice were caught in larger numbers by day. Larger numbers of other flatfish, greysole, yellowtail, and winter flounder, were taken in night tows.

Diurnal difference in size composition was examined for cod, haddock, yellowtail, and American plaice. More small haddock (less than 35 cm) were caught by day than by night. Catches of large haddock tended to be larger at night. For cod, size composition of daytime hauls was not significantly different from that at night. There was some suggestion that more small yellowtail (less than 22.5 cm) were taken in night than day tows. No significant diurnal difference was found for large fish. Size composition of plaice in day tows did not vary significantly from those at night.

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The *Coregonus autumnalis* Complex in Alaska and Northwestern Canada[1,2]

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ABSTRACT

The systematic status of ciscoes of the *Coregonus autumnalis* complex in Alaska and northwestern Canada is discussed. Two forms of "*autumnalis*" ciscoes occur in this area: a form with 26–30 gill rakers on the lower limb of the first gill arch is found in the Beaufort and Chuckchi Seas, and a form with 21–25 gill rakers on the lower limb of the first gill arch is found in the Gulf of Alaska, the eastern Bering, and the Chuckchi Seas. Both forms occur along the arctic coast of Alaska, apparently without intergrading, and therefore are considered valid species. The form with the high gill raker number is *Coregonus autumnalis* (Pallas) (*sensu stricto*), and that with the low gill raker number is *Coregonus laurettae* Bean.

INTRODUCTION

The ciscoes, or "freshwater herrings," are a holarctic group of pelagic whitefishes distinguished by a large, superior, or terminal mouth and many gill rakers. Most North American authors (Koelz, 1929; Dymond, 1943; Smith, 1957) consider the ciscoes a separate genus, *Leucichthys*, whereas European authors (Berg, 1948; Svardson, 1957) include the ciscoes in the genus *Coregonus*. Norden (1961) demonstrated that the antrose upper jaw and large number of gill rakers, supposedly diagnostic for *Leucichthys*, have evolved independently in a lacustrine species of *Prosopium* (*P. gemmiferum*). Therefore, these characters are not diagnostic for *Leucichthys*, and I follow Norden (1961) and Lindsey (1962) in including the ciscoes in the genus *Coregonus*.

Early authors (Dall, 1871; Bean, 1882; Anderson, 1913) recognized two distinct types of ciscoes in the coastal areas of the eastern Bering and Beaufort Seas. These types differed in size, position of the mouth, and color of the ventral fins. In Alaska, Bean (1882, 1889) named the larger form with a terminal mouth and immaculate ventral fins *Coregonus laurettae*, and the smaller form with a superior mouth and dusky ventral fins *Coregonus pusillus*. Scofield (1899) named a third species of cisco, *Argyrosomus alascanus*, from Point Hope, Alaska.

Dymond (1943) considered the two kinds of ciscoes in the Mackenzie Delta region identical to the Alaskan species named by Bean, but suggested that the latter species (*C. laurettae* and *C. pusillus*) are conspecific with two Siberian ciscoes, *Coregonus autumnalis* (Pallas) and *Coregonus sardinella* Va-

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lenciennes, which have priority. Walters (1955) concurred with Dymond's synonymies, and further suggested that *Argyrosomus alascanus* and *Coregonus subautumnalis* Kaganowsky (known only from the Penzhina River, Sea of Okhotsk drainage) are also synonymous with *C. autumnalis*.

The present study deals with ciscoes of the "*autumnalis*" type (*A. alascanus*, *C. autumnalis*, and *C. laurettae*) from the coastal areas of Alaska and northwestern Canada, and is an attempt to determine the systematic status of these forms. Ciscoes of the *autumnalis* type are readily separable from other sympatric ciscoes on the basis of mouth position, dorsal fin shape, and ventral fin color (see Walters, 1955; McAllister, 1961).

MATERIALS AND METHODS

This study is based on 132 specimens from the following localities in Alaska, the Yukon Territory, and the Northwest Territories: Point Barrow (USNM 27965), Elson Lagoon (SU 49174, UMMZ 174546, ROM 18479–18496), Beechy Point (SU 49074), Oliktok (SU 49065), Herschel Island (NMC 58-130, 136, 137, and NMC 60-465), Beaufort Sea (BC 55-40), Tuktoyaktuk (BC 53-282), Aklavik (ROM 15766-15785), Peel River (BC 60-346), Liverpool Bay (uncataloged, Fisheries Research Board of Canada), Baillie Island (uncataloged, Fisheries Research Board of Canada), Lake Namaycush, Victoria Island (uncataloged, Fisheries Research Board of Canada), Bathurst Inlet (BC 61-358), Point Hope (BC 60-486, SU 5611), Eschsholtz Bay (USNM 29935, 29947), Port Clarence (USNM 27915), Nome Beach (BC 60-378), Safety Sound (BC 60-377), Unalakleet (BC 60-385), Andreafsky (BC 60-392), Nulato (USNM 29902), Snag Point (BC 60-455), Ship Creek (SU 41858).

Individuals ranged from 40 to 380 mm in standard length, and were from both marine and fresh waters. The specimens are now in the museums of the Institute of Fisheries at the University of British Columbia (BC), The Royal Ontario Museum (ROM), Stanford University (SU), the Museum of Zoology at the University of Michigan (UMMZ), the National Museum of Canada (NMC), and the U. S. National Museum (USNM).

One character, the number of gill rakers on the lower limb of the first gill arch, was found to be of major taxonomic significance. Gill rakers were counted by dissecting out the left gill arch and counting the rakers under a binocular microscope. Other characters examined were: the number of pored lateral line scales, the number of pyloric caeca, and the number of fin rays. All counts and measurements follow Hubbs and Lagler (1958).

There is considerable variation in length composition and sex ratio between different collections. Therefore, the number of gill rakers on the lower limb of the first arch was tested for correlation with length, and for sexual dimorphism. Correlation was tested using the correlation coefficient (Snedecor, 1956), and sexual dimorphism by the *t*-test. The level of significance is 5%.

The number of gill rakers on the lower limb of the first arch is not significantly correlated with standard length ($p > 0.10$), and does not show sexual dimorphism ($p > 0.10$).

RESULTS

The frequency distribution of the number of gill rakers on the lower limb of the first arch for all ciscoes, of the *autumnalis* type, examined (Fig. 1) is distinctly bimodal. On this basis, all of the *autumnalis* ciscoes were separated into two groups: individuals with 25 or fewer gill rakers on the lower limb, and individuals with 26 or more gill rakers on the lower limb. The two groups of *autumnalis* ciscoes, so divided, also differ significantly ($p < 0.001$) in mean numbers of lateral line scales and pyloric caeca (Table I). However, the overlap in the ranges of these characters limits their usefulness in identifying individuals.

The geographic distributions of these two groups of *autumnalis* ciscoes are almost completely allopatric. They only occur together along the arctic coast of Alaska where they appear to intergrade (Fig. 2).

Figure 3 presents the gill raker frequency distributions for the two apparently intermediate collections in Fig. 2. These frequency distributions are also bimodal, and suggest that the two groups of *autumnalis* ciscoes do not intergrade along the arctic coast of Alaska, but exist sympatrically and therefore should be considered as valid species.

DISCUSSION

NOMENCLATURE

Pallas (1776) described *Salmo* (*Coregonus*) *autumnalis*, the "omul," from the Pechor and Enissei Rivers of western Siberia. The original description is inadequate to determine which of the two North American species of *autumnalis* ciscoes corresponds to *Coregonus autumnalis*. However, recent Russian descriptions (Berg, 1948; Andriashev, 1954) of *C. autumnalis* indicate the

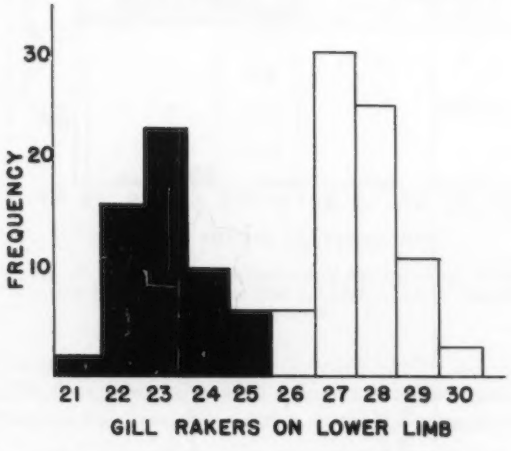

FIG. 1. Frequency distribution of number of gill rakers on the lower limb of the first gill arch, for the entire sample of *autumnalis* ciscoes examined.

TABLE I. Number of gill rakers on the lower limb, pyloric caeca, and pored lateral line scales for the entire sample of *autumnalis* ciscoes examined. Only gill rakers were counted on all specimens.

	Number gill rakers on lower limb																			
	21		22		23		24		25		26		27		28		29		30	$\bar{X}$
C. autumnalis											6		30		25		12		3	27.68
C. laurettae	2		16		23		9		6											23.01

	Number pyloric caeca																			
	71 76	77 82	83 88	89 94	95 100	101 106	107 112	113 118	119 124	125 130	131 136	137 142	143 148	149 154	155 160	161 166	167 172	173 178	179 184	$\bar{X}$
C. autumnalis								2	4	5	6	2	4	4	5	–	1	–	1	142.0
C. laurettae	1	2	4	8	5	6	8	3	1											102.4

	Number of pored lateral line scales													$\bar{X}$
	75 77	78 80	81 83	84 86	87 89	90 92	93 95	96 98	99 101	102 104	105 107	108 111		
C. autumnalis			1	1	3	5	5	19	4	4	2	1		96.6
C. laurettae	1	4	5	12	7	1	3							85.2

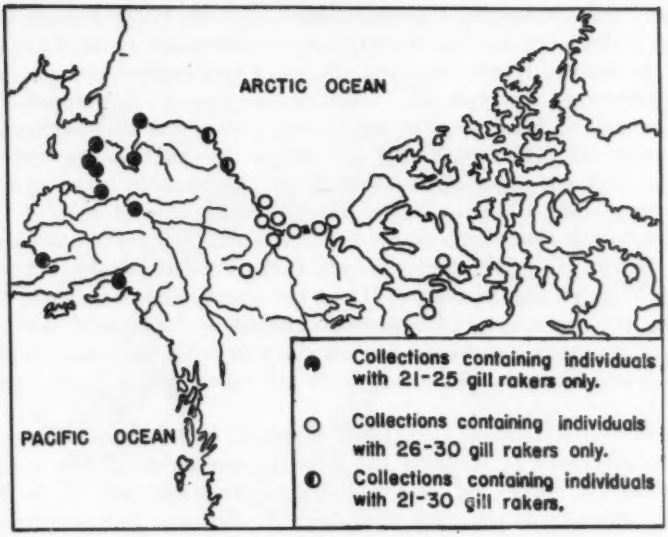
ARCTIC OCEAN
PACIFIC OCEAN
Collections containing individuals with 21-25 gill rakers only.
Collections containing individuals with 26-30 gill rakers only.
Collections containing individuals with 21-30 gill rakers.

FIG. 2. Known North American distribution of the two groups of *autumnalis* ciscoes.

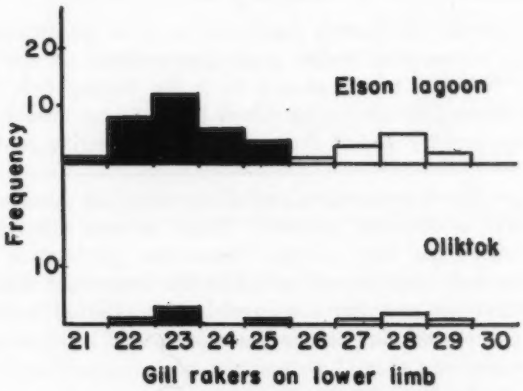

FIG. 3. Frequency distributions of number of gill rakers on the lower limb of the first gill arch in arctic Alaskan populations.

omul of Siberia has a total of 35–51[3] (usually 42–46) gill rakers on the first arch. The North American form with a high gill raker number has a total of 41–46 gill rakers on the first arch, and is probably conspecific with *Coregonus autumnalis*.

[3]Since the Russian descriptions list only the total number of gill rakers on the first gill arch, this character must be used in comparing the Eurasian and North American forms.

Bean (1882) described *Coregonus laurettae* from Point Barrow and Port Clarence, Alaska. Again, the description is inadequate to determine which, if either, of the two North American species of *autumnalis* ciscoes is *Coregonus laurettae*. However, the types of *C. laurettae* are in the U. S. National Museum. Unfortunately, no holotype was designated, and of the three syntypes from Point Barrow (USNM 27695), two are the low gill raker number species, and one is the high gill raker number species (*C. autumnalis, sensu stricto*). The syntype from Port Clarence is the low gill raker number species (14 + 23 gill rakers on the lower limb of the first gill arch). Because of this situation, I select the low gill raker specimen from Port Clarence, Alaska, bearing U. S. National Museum tag number 27915 as the lectotype of *Coregonus laurettae*. Therefore, the two species of *autumnalis* ciscoes in Alaska and northern Canada are: *Coregonus autumnalis* (26–30 gill rakers on the lower limb of the first arch), and *Coregonus laurettae* (21–25 gill rakers on the lower limb of the first arch).

Jordan and Evermann (1911) and Dymond (1943) suggest that *Argyrosomus alascanus* and *C. laurettae* are probably synonymous. The holotype of *A. alascanus* (SU 5611) has 21 gill rakers on the lower limb of the first gill arch. This strengthens the contention that *A. alascanus* is a synonym for *C. laurettae*.

Distribution and Origin of *Coregonus autumnalis* and *C. laurettae*

Figure 4 presents the known distributions of *C. autumnalis* and *C. laurettae*. *Coregonus autumnalis* ranges from Mezen Gulf on the White Sea to Bathurst Inlet, N.W.T., but is absent from the Bering Sea. *Coregonus laurettae* ranges from the Gulf of Alaska (Cook Inlet) to the mouth of the Colville River, but is apparently absent from the western Bering and the Beaufort Seas.

Morphologically, *C. autumnalis* and *C. laurettae* are similar and are probably derived from a common ancestor. Their present distribution (Fig. 4) suggests that before the start of the Pleistocene glaciations, this ancestral form was continuously distributed in the Arctic Ocean and Bering Sea. With the onset of glaciation and the emergence of the Bering Land-bridge, it is suggested that this continuous population was divided into two geographically isolated populations, and that *C. autumnalis* then evolved in the Arctic Ocean and *C. laurettae* in the Bering Sea.

If *C. laurettae* evolved in the Bering Sea, its absence from the Asian coast of the Bering Sea is particularly puzzling unless *C. subautumnalis* represents a western Bering derivative of *C. laurettae*. Walters (1955) suggested that *C. subautumnalis* is a synonym of *C. autumnalis*. However, this suggestion is based on the erroneous assumption that *C. autumnalis* and *C. laurettae* are also synonymous. The true relationship of *C. subautumnalis* to *C. autumnalis* and *C. laurettae* cannot be determined on the available data.

Note added in proof. While this paper was in press, Kurenkov and Ostroumov (1965) reported *C. sardinella* from Kamchatka. However, the gill

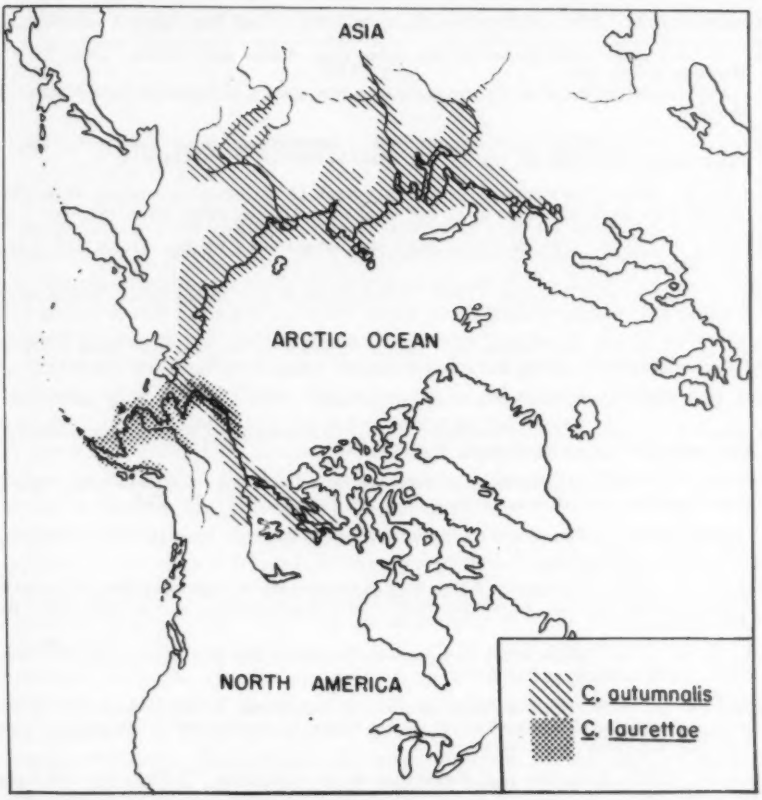

FIG. 4. The known geographic ranges of *Coregonus autumnalis* and *C. laurettae*. The Eurasian range for *C. autumnalis* is derived from Berg (1948) and Andriashev (1954).

raker counts that they give (30 and 32) are too low for *C. sardinella* in the Bering Sea region (41–53 is typical for *C. sardinella* in the eastern Bering Sea), and it is probable that their specimens represent *C. laurettae*.

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NOTES

Alkaline Phosphatase in Fish Scales[1,2]

The occurrence of alkaline phosphatases, enzymes associated with the formation of bone, with scales of fish was reported many years ago. Their possible use in studying growth of fish was apparent to Roche et al. (1940). These workers studied the phosphatase activity of aqueous solutions containing fragmented organs of fish, including scales of adult sardines, *Clupea pilchardus* Walbaum, from the Mediterranean Sea. They found greater relative phosphatase activity with scales taken during spring and summer when fish were growing than with scales taken during the rest of the year.

However, Wallin (1957), after a thorough examination of scales of roach, *Rutilus* sp., reported that neither in connection with normal nor with regenerated scales, could alkaline phosphatase be demonstrated in connective tissue, active cells, or scale matrix. Also, he could not find phosphatase activity in scales of the guppy, *Lebistes* sp., but did find it in the epidermis of regenerating roach scales. From a series of experiments he concluded that fish scales represent a simplified kind of bone that is formed without the assistance of an alkaline phosphatase, and furthermore, that in previous work in which such enzymes were reported to be present in scales, they were really in epidermis.

In some recent work, mostly with fish occurring in the Great Lakes, alkaline phosphatase activity was found in the epithelium of scales of rock bass, *Ambloplites rupestris* (Rafinesque). It was not found in the scales themselves nor in the epithelium of scales of all the fish other than rock bass listed in Table I.

Gomori's method (Pearse, 1960) is commonly used for the detection of alkaline phosphatase activity in tissues. During incubation of tissue sections or scales in an organic phosphate medium containing calcium and magnesium ions, calcium phosphate is deposited at the sites of the enzymes in the tissue. When the tissue is then treated with a cobaltous salt, cobalt ions attach to the newly deposited calcium phosphate. A subsequent treatment with ammonium or sodium sulfide results in black deposits of cobalt sulfide in the tissue.

In his work, Wallin (1957) used Gomori's method with several variations. He also used another method of studying scales that he called Stoeltzner's reaction. Stoeltzner (1905) had found that when tissue slices were treated simply with cobalt nitrate and then ammonium sulfide, excellent selective blackening of calcified material resulted.

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[1]Contribution No. 65-19 of the Ontario Dept. of Lands and Forests, Research Branch, Maple, Ont.

[2]Based on a thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy at the University of Toronto.

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TABLE I. Species from which scales were taken and treated by Gomori's and Stoeltzner's methods.

Alewife	*Alosa pseudoharengus* (Wilson)
Cisco	*Coregonus artedi* Le Sueur
Lake whitefish	*Coregonus clupeaformis* (Mitchill)
Round whitefish	*Prosopium cylindraceum* (Pallas)
Hybrid trout	*Salvelinus namaycush* (Walbaum) × *S. fontinalis* (Mitchill)
American smelt	*Osmerus mordax* (Mitchill)
Northern pike	*Esox lucius* Linnaeus
Spottail shiner	*Notropis hudsonius* (Clinton)
Carp	*Cyprinus carpio* Linnaeus
White sucker	*Catostomus commersoni* (Lacépède)
Longnose sucker	*Catostomus catostomus* (Forster)
White bass	*Roccus chrysops* (Rafinesque)
Rock bass	*Ambloplites rupestris* (Rafinesque)
Smallmouth bass	*Micropterus dolomieui* Lacépède
Yellow perch	*Perca flavescens* (Mitchill)
Walleye	*Stizostedion vitreum vitreum* (Mitchill)
Freshwater drum	*Aplodinotus grunniens* Rafinesque
Tilapia	*Tilapia mosambica*

Hence, Stoeltzner's reaction (or method) is like Gomori's method for the detection of alkaline phosphatase in that through the use of the same salt solutions, cobalt nitrate and ammonium sulfide, calcified material is darkly stained. Stoeltzner's method differs from Gomori's in that the incubation step is omitted; therefore, Stoeltzner's method does not detect the presence of alkaline phosphatase.

Wallin (1957) identified and named three zones of the bony layer of scales that are differentiated by Stoeltzner's reaction (Fig. 1). At the extreme edge of the scale is a clear zone of uncalcified osseoid tissue. Immediately inside of it is the darkly stained zone of calcification followed by the remainder of the scale which is completely calcified and more lightly stained. Thus Wallin was able to define the growing area of the bony layer of scales more closely than just the margin, it being the osseoid zone and the zone of calcification.

Scales taken in summer from the fish listed in Table I were treated by Gomori's method. With the exception of rock bass, all scales had the appearance of that shown in Fig. 1, in particular a clear osseoid zone was visible at the margin. Scales of rock bass had a wide, very dark band extending to the extreme

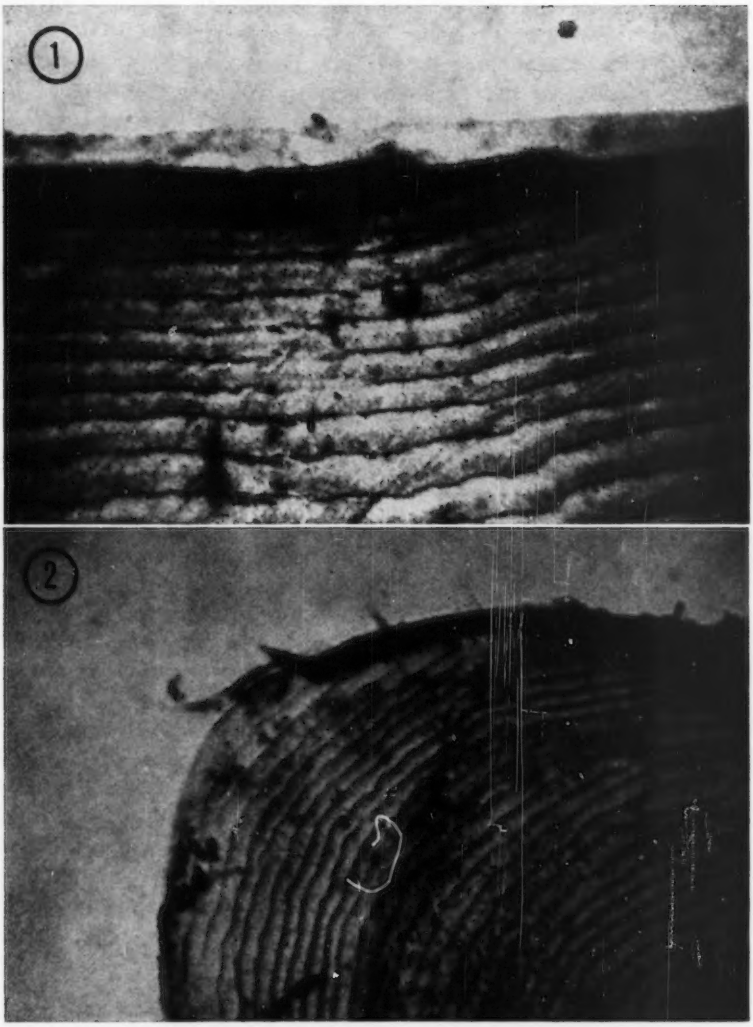

FIG. 1. Lateral portion of a white sucker scale treated according to Stoeltzner. Magnification approximately × 100. The three zones of the bony layer of the scale identified by Wallin (1957) are visible. At the top is the clear osseoid zone, immediately below it is the narrow, very dark zone of calcification, followed by the more lightly stained, completely calcified remainder of the scale in which circuli are visible.

FIG. 2. Anteriolateral corner of a rock bass scale treated by Gomori's method. Note the clear osseoid zone beneath the torn piece of darkly stained epithelium extending out from the edge of the scale. Magnification approximately × 100.

edge of the scales, hence, instead of being clear, the osseoid zone appeared to be darkly stained. When scales from all these fishes, including rock bass, were treated according to Stoeltzner, they all had the same appearance, that of Fig. 1.

The darkness of the osseoid zone of rock bass scales treated by Gomori's method, and the fact it was clear in scales treated according to Stoeltzner, indicates the presence of an alkaline phosphatase. The same enzyme does not appear to be present with scales of the other fishes because they had the same appearance whether treated by a method that would detect alkaline phosphatase (Gomori) or by a method that would not detect it (Stoeltzner).

It seemed so remarkable that alkaline phosphatase activity would only be present with the scales of one species out of the 18 examined, that the following experiment was carried out. The temperature of boiling water (as well as solutions of very low osmotic pressure) is known to denature enzymes. Eight scales were taken from a rock bass, four were treated by Gomori's method and four by Stoeltzner's method. Immediately before the treatment, two scales in each group were placed in boiling distilled water for 1 min. After treatment the scales were mounted on slides in Canada Balsam.

In both groups of scales treated according to Stoeltzner, a zone of calcification and an osseoid zone were evident. On gross examination the completely calcified part of the scale appeared slightly darker in boiled scales treated by either method. In incubated scales there was a light osseoid zone outside of a dark zone of calcification (as above) in the boiled scales, while the nonboiled ones exhibited the typical very dark, wide band extending to the scale edges.

A quantitative measure of the extent of the darkening was obtained by determining the optical density of small portions of the osseoid zone of these scales. (Optical density is a measure of the opacity of a substance; the darker or more opaque it is, the higher its optical density.) Optical density determinations from the eight scales are shown in Table II. The manner in which these measurements were obtained, as well as the exact procedures followed in the treatment of the scales by Gomori's and Stoeltzner's methods, were described by Coble (MS, 1965). It is evident in the data that, with scales treated by Gomori's method without a denaturation treatment, the osseoid zone was much darker than that of the other scales. In the boiled incubated scales the osseoid zone

TABLE II. Optical densities of the osseoid zone of eight rock bass scales treated as indicated.

Not incubated (Stoeltzner)		Incubated (Gomori)	
Boiled	Not boiled	Boiled	Not boiled
0.12	0.08	0.37	1.29
0.12	0.17	0.34	1.34

was not uniformly darker than in both groups of nonincubated scales as might be suggested by the densities, rather, it had a peppered appearance.

These results indicate that an alkaline phosphatase is indeed present in rock bass. When a tissue containing such an enzyme is treated by Gomori's method, reactions occur which cause a particular appearance, and rock bass scales thus treated take on this appearance. If they are first treated in a manner that would denature an enzyme, they do not take on that appearance. When they are treated by another method that produces a different appearance, it is not altered by the same denaturation treatment.

Figure 2 is of use in answering the question whether the alkaline phosphatase occurs in the scale itself or in the epithelium of rock bass scales. The photograph shows the anteriolateral corner of a rock bass scale treated according to Gomori. It can be seen that the dark epithelium ends right at the corner. Beneath the torn piece of epithelium extending out from the scale edge can be seen the clear osseoid zone. It is evident that the epithelium clings tightly to the scale to give the effect that the enzyme is present in the scale itself.

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Underwater Observations on Fish in an Off-Bottom Trawl

Scuba-equipped scientists of the U. S. Fish and Wildlife Service, Bureau of Commercial Fisheries, Exploratory Fishing and Gear Research Base, Seattle, Wash., recently made underwater observations on fish within the influence of an off-bottom trawl (Fig. 1). The observations were incidental to the major objective of trawl design evaluation; however, they provided an opportunity to further our knowledge of fish behavior. The trawl is an experimental wing type, having an 80-fath headrope and footrope. Hydrofoil otterboards spread the wings to more than 150 ft and provide a vertical mouth opening of 16–20 ft. The mesh size is 3-inch stretch measure.

More than 160 dives were made during the underwater study to diving depths between 30 and 100 ft in Puget Sound, Wash. Objects were distinguishable underwater at distances of 15 to over 50 ft during the study.

Techniques. Observations were made with the divers hanging on the trawl. This technique permitted easy access to all parts of the net, both inside and out. Divers made contact with the moving trawl by pulling themselves down a buoy line tied to the wing tip. A scuba tank provided enough air to remain underwater for periods up to 22 min. Measurements and pertinent observations were recorded on a small piece of opaque cellulose acetate glued to the diver's wet suit arm.

Herring behavior. Small schools of 6-inch herring (*Clupea pallasii*) were observed on several occasions. The schools were usually noticed after they had entered the net and were either in the body of the net just ahead of the codend or within the codend. Although the herring could easily pass through the web, they did not readily do so. The herring tended to maintain a position relative to a specific area of the web; yet, occasionally, they would swim rapidly forward 3 or 4 ft to assume a new position. Several schools swam forward out of the codend and continued beyond the range of visibility (about 15 ft) toward the trawl mouth. During two periods of observations, schools outside the net approached and oriented to the web. Individuals broke from the outside school and swam through the meshes into the bag to join those within.

Some herring schools reacted to sudden movements. The rapid release of air bubbles from scuba units often caused the fish to scatter in all directions through the meshes.

Herring swam within the trawl for periods up to 20 min. Flow measurements taken at the net indicated the speed of the trawl through the water was over 2 knots. The current loss inside the codend was less than 10% as indicated by water flow measurements. Herring were observed maintaining position within the trawl at all points from just inside the mouth to the "bitterend" of the bag. This suggests these fish can maintain a swimming speed of at least 1.13 m/sec (3.7 ft/sec) for 20 min or more.

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Other species. Evidence of fish swimming out of the trawl was noted. On four separate occasions, individual fish (dogfish, *Squalus acanthias*; sablefish, *Anoplopoma fimbria*; and silver salmon, *Oncorhynchus kisutch*) were observed in the codend but escaped before the trawl was retrieved. In each instance no other fish were seen or captured in the net. In the two instances when individual dogfish were seen, they were first noticed being held against the "bitterend" of the bag by water pressure. They remained in this position probably less than 5 min. When pushed free of the web by the divers, the fish swam ahead 4 or 5 ft and appeared to orient to a section of the codend web and the net speed. The silver salmon swam about 4 ft from the web at the junction of the trawl body and codend. As the fish was approached from behind, it quickly swam forward toward the net mouth beyond the range of visibility. The sablefish swam into the current within the codend and maintained its position despite the presence of divers outside the codend. Observations were limited to a minute or two.

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(Fig. 1 opposite)

FIG. 1. *Top left.* Underwater observers hang onto the trawl while watching fish and net action. *Bottom left.* Scuba-equipped scientist shown stationed on trawl floatline to observe reaction of fish within trawl's influence. *Top right.* Underwater view showing diver using a flow meter to measure trawl speed through the water. *Bottom right.* Herring shown inside the trawl bag swimming along at the net speed.

Free Fatty Acid Formation in Cod Livers Sterilized by Irradiation

There has been some uncertainty as to the origin and significance of the lipolytic agents responsible for the production of free fatty acids in cod oil (sun rotted). Both cod liver and bacterial enzymes have been suspected (Brocklesby, 1941; Johnston, 1941). The presence of putrid odours in stored livers, and a vigorous fermentation reaction in liver foots (Drummond and Hilditch, 1930), along with observations on the effect of antibacterial agents (Guttmann and Vandenheuvel, 1958; Jangaard and Power, unpublished data), would tend to place emphasis on a bacterial origin for the lipases, although it is well known that lipolytic enzymes are also present normally in cod livers. It is possible under some commercial conditions where fresh livers may be added to livers already rotting, that there would be a substantial contribution of lipolytic enzymes from the high levels of bacteria which would be present under these conditions. These bacteria undoubtedly aid in the liberation of the oil from the liver cells.

We have now shown by gamma irradiation sterilization of fresh cod livers that nonbacterial agents (presumably lipolytic enzymes) normally present in cod livers remain fully active after irradiation and produce the same levels of free fatty acids as similar samples not irradiated, and heavily contaminated with bacteria.

Normally tissue, such as liver, when removed from the body cavity under clean (not aseptic) operating conditions is only slightly contaminated with bacteria. A dosage of 0.5 million rads was therefore adequate to produce sterile conditions. A dosage of 1 million rads did not appear to affect the lipolytic agents.

It should also be remarked that liberation of the oil from the liver cells did not take place to any extent in the irradiated samples and is, therefore, not a prerequisite for free fatty acid formation.

Cod (*Gadus morhua*) were taken at Terence Bay, N.S., and transported live to the laboratory in Halifax. The fish were killed, the livers removed under clean but not sterile conditions, and normally held for up to 4 hr at room temperature before further treatment. The individual livers, free of bile sacs, were chopped to pea-size chunks. Three 50-g portions from the liver under examination were placed in cork-stoppered standard oil sample bottles (4-oz capacity). Two bottles received, respectively, 1 and 0.5 million rads in a Model GC-220 Gammacell[1] utilizing a 15,000 c source of cobalt 60, and the third bottle served as a control. The bottles were kept at room temperature (ca. 25 C) for 7 days. Following this period, in three cases the lipids were recovered by solvent extraction and examined for free fatty acids (Table I).

[1]Manufactured by the Atomic Energy of Canada, Ltd., Ottawa, Ontario.

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TABLE I. Per cent free fatty acid (calculated as oleic acid) in oil recovered from irradiated cod livers and controls after 7 days.

	Irradiation treatment		
Liver no.	1 million rads	0.5 million rads	None (control)
1	22.0	23.5	25.6
2	14.6	13.9	10.1
3	11.7	13.7	13.0

In the fourth case, the bottles were shaken vigorously and the contents aseptically removed for qualitative bacteriological examination. Difco[2] Brain Heart Infusion, Difco Liver Infusion, Difco Todd-Hewitt and BBL[3] Tripticase Soy media were used in both solid and liquid form for this purpose. The results indicated that the irradiated samples were sterile while the nonirradiated sample (control) was very heavily contaminated with many different bacterial genera.

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[2]Difco Laboratories, Inc., Detroit, Michigan.
[3]Baltimore Biological Laboratory, Division of B-D Laboratories, Baltimore, Maryland.

Downstream Movements of Trout Fry (*Salmo trutta*) in a Dartmoor Stream

During work on drift invertebrates in a moorland stream on Dartmoor, southwest England (Elliott, 1965, and unpublished Ph.D. thesis), fry (ca. 2.5 cm) of *Salmo trutta* L. were taken in the drift samples of March and April 1964. The bionomics of the brown trout population have been investigated by Horton (1961). The stream studied, the Walla Brook, is slightly acid (pH 6.4–6.8), with a low water temperature (rarely above 15 C or below 4.5 C). Daily fluctuations in water temperature often occurred, these being 3.4 C and 3.2 C for the sampling periods in March and April, respectively (Tables I and II). At the sampling point the stream bottom was chiefly small stones and gravel with *Myriophyllum spicatum* L. as the dominant aquatic

TABLE I. Number of fry per 3-hr sample, March 15–16, 1964.

	Sample number							
	1	2	3	4	5	6	7	8
Emptying time (*hr*)	15	18	21	24	3	6	9	12
Water temperature (*C*)	8.2	8.2	6.4	4.8	4.8	4.8	6.0	6.8
Surface catch	0	0	14	4	3	1	2	0
Midwater catch	0	0	16	6	5	2	0	0
Total fry per 3 hr	0	0	30	10	8	3	2	0

TABLE II. Number of fry per 3-hr sample, April 12–13, 1964.

	Sample number							
	1	2	3	4	5	6	7	8
Emptying time (*hr*)	15	18	21	24	3	6	9	12
Water temperature (*C*)	8.6	9.1	8.6	8.0	7.4	6.8	8.4	10.0
Surface catch	0	1	15	21	13	12	0	1
Midwater catch	0	0	15	27	19	8	0	0
Total fry per 3 hr	0	1	30	48	32	20	0	1

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plant. The width was about 160 cm with an average depth of 25 cm, thus the cross-sectional area of the stream at the sampling point was about 4000 cm^2. Drift sampling was both at the surface and in the midwater region, using one surface net and three midwater samplers at the same cross-section of stream. The total sampling area was 579 cm^2 or about one-seventh of the total cross-sectional area. All sampling was from midday (1200 hr Greenwich Mean Time) to the following midday, the four nets being emptied every 3 hr to give eight samples per 24 hr.

In March, the periods of day and night were equal with four samples in each (sunset 1800 hr GMT, sunrise 600 hr GMT). The surface net sampled 243,000 litres/3 hr and the three midwater nets a total of 160,000 litres/3 hr, the average velocity of the stream being 0.60 m/sec. Only two fry were taken in the four day samples contrasted with 51 fry in the night samples, 30 being taken in sample 3 alone (Table I).

In April, night and day periods were almost equal (sunset 1900 hr GMT, sunrise 500 hr GMT), but it was dark for only 2 hr of samples 3 and 6. The surface net sampled 188,500 litres/3 hr and the three midwater nets a total of 124,000 litres/3 hr, the average velocity of the stream being 0.45 m/sec. Although less water was sampled, the number of fry was greater than for March, 130 being taken in the four night samples and only two in the day samples (Table II). The greatest number was taken in sample 4, this probably being due to the longer night sampling time of 3 hr compared with only 2 hr for sample 3.

The catches in both March and April indicated that downstream movement of fry occurred chiefly at night, being at a maximum in the early hours of the night. During both these months the daily mean water temperature was below 14 C, the critical temperature below which downstream movement occurred for fry of *Salmo gairdneri* L. (Northcote, 1962). The onset of the movement appeared to be associated with the sunset, when the light intensity at the surface of the water was 0.02 ft-c. This is also in agreement with the observations of Northcote (1962) on fry of *Salmo gairdneri*, whilst several workers have observed similar behaviour in Pacific salmon fry (Brett and Ali, 1958; Dvinin, 1959; Hoar, 1951, 1954, 1956; Mackinnon and Brett, 1955; McDonald, 1960; Neave, 1955).

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